HAYATI
Journal of Biosciences

Vol. 30 No. 2, March 2023 302-312
DOI:10.4308/hjb.30.2.302-312
ISSN: 1978-3019
EISSN: 2086-4094

Growth of Red Amaranth (*Amaranthus cruentus* L.) Cultivated on Soil-Based Substrate Amended with a Residue of the Black Soldier Fly Larvae Containing Heavy Metals

Hana Haruna Putri[1], Benyamin Lakitan[2,3*], Zaidan Panji Negara[2], Strayker Ali Muda[2]

[1]*Graduate School of Agriculture, Forestry, and Fisheries, Kagoshima University, Korimoto 1-21-24, Kagoshima, 890-0065, Japan*
[2]*College of Agriculture, Universitas Sriwijaya, Indralaya, Indonesia*
[3]*Research Center for Sub-optimal Lands, Universitas Sriwijaya, Bukit Besar, Palembang, Indonesia*

ARTICLE INFO

Article history:
Received February 11, 2022
Received in revised form August 1, 2022
Accepted September 27, 2022

KEYWORDS:
Hermetia illucens,
leafy vegetable,
organic ameliorant,
stunted growth,
toxic element

ABSTRACT

Red amaranth (*Amaranthus cruentus* L.) is a fast-growing and nutritious leafy vegetable. The seed population density needs to be known appropriately to obtain the optimal yield of marketable sizes plants. The residue of black soldier fly (BSF) larvae culture has been recognized as a potential source of nutrients for cultivating red spinach plants. This study was designed to search for the best combination of plant population density and BSF concentration to obtain the optimal marketable yield of the red amaranth plant. In addition, an accurate leaf area estimation of the red amaranth plant was also developed using leaf dimensions as a predictor with several regression models. Two experiments were conducted. Firstly, the BSF residue was applied at a high rate (up to 50%) using a floating culture system. Application of the residue at the rate of 30% and higher significantly inhibited the growth of the red amaranth. Secondly, the application rate was reduced to 10% and 20%, and the experiment was conducted using the conventional cultivation system. Results indicated that the optimum application rate of the BSF residue was 10% for enhancing growth. Application of seed density at 30 mg/dm^2 produced an optimum marketable yield of the red amaranth since a higher population density causes plant-plant competition. Thus, creating high size variability within the population or reducing the average size of the harvested plants. Lastly, the leaf area of red amaranth can be accurately estimated using the leaf length x width (LW) as a predictor using the zero-intercept linear regression model.

1. Introduction

Red amaranth (*Amaranthus cruentus* L.) is a leafy vegetable with red velvet color, rich in nutrients, minerals, and antioxidants. Nutrient contents in red amaranth are richer than those in green (Sarker and Oba 2019). For instance, this leafy vegetable contains betalain pigments that have potential application as a natural colorant in the food industry, functional foods, and various pharmacological substances (Gengatharan *et al.* 2015).

The red amaranth is harvested during the vegetative phase based on size, not by the age of the plant. Amaranth is also a fast-growing vegetable (Onyango *et al.* 2012) that can be harvested within a month if sufficient essential nutrients are available. However, in most cases, nutrient availability has been the limiting factor in vegetable cultivation (Lakitan *et al.* 2018, 2019), including the red amaranth. Organic fertilizer is preferred since it provides nutrients and improves soil's physical properties. Black soldier fly (BSF) residue is expected to fulfill these needs, at least partially. The BSF residue has not been widely used as organic fertilizer.

The BSF culture produces maggots as a protein source for poultry feed (Barragan-Fonseca *et al.* 2017). It enhances the decomposition of an unused and large quantity of empty fruit at oil palm plantations (Dickinson *et al.* 2019). BSF larvae are commonly used as an alternative feed for livestock because they contain high protein. The larvae can be fed a wide range of organic waste, i.e., poultry waste (Cickova *et*

* Corresponding Author
E-mail Address: blakitan60@unsri.ac.id

al. 2015) and palm oil kernel (Bokau and Witoko 2017). The life cycle of BSF unavoidably released waste that could be used as manure. The latest research on BSF manure application on rice (Wu *et al.* 2020), pepper and shallot (Quilliam *et al.* 2020), maize (Tanga *et al.* 2021), and Swiss chard (Chirere *et al.* 2021) shows positive results on yield. It also discovered that BSF manure could reduce dead cowpea plants due to Fusarium wilt outbreaks (Quilliam *et al.* 2020).

The organic fertilizer resource with high nutrients is rarely found. Therefore, organic fertilizer needs to be applied in large quantities. Based on Zhu *et al.* (2015), BSF manure contains 3,36% N-nutrient with a pH of 7.78, so such high nutrient content could be a promising organic fertilizer for red amaranth cultivation. Meanwhile, red amaranth, as a leafy vegetable, could be a model for analyzing leaf growth parameters. On that account, this research aims to open the possibility of using the BSF residue as a source of nutrients to increase yield in red amaranth and develop a non-destructive yet accurate leaf area estimation model.

2. Materials and Methods

2.1. Plant Material and Growing Media

Red amaranth used in this research was Mira variety. This variety adapts well to a tropical lowland ecosystem. The soil-based growing substrate was mixed with residue produced by black soldier fly (*Hermetia illucens* L.). The results of the content analysis of the two growing media are shown in Table 1. The mixed substrate was bucketed into plastic pots 20 cm from the base. The plastic pots' dimension was 27.5 cm in inner-upper diameter and 20.0 cm in inner-base diameter. The residue was collected as solid waste produced at BSF maggot culture in oil palm plantations.

2.2. Experiment 1: Composition of BSF Residue in the Floating Culture System

The first experiment aimed to determine the optimum application rate of BSF residue for the red amaranth cultivated using the floating culture system. The first experiment was arranged based on the Randomized Block Design, with the treatments consisting of 4 concentrations of BSF residue (0%, 10%, 30%, and 50%, v/v) mixed with soil-based media. Three free-floating rafts were placed in a concrete experimental pool filled with water. The raft used was a modified version of a patented raft of Lakitan and Siaga (Patent No. IDP 000065141, granted on 10 December 2019). Each raft was assigned as a block. Details on the design, materials used, and technical specifications of the raft were previously described in Siaga *et al.* (2018, 2019). Fifteen plastic pots with a 27.5 cm upper diameter and 4 bottom holes were placed on each raft. The pots were loaded with the mixed substrate until the bottom holes were in direct contact with the water surface, i.e., ensuring water penetration upward into the substrate occurred due to capillarity force.

The red amaranth was germinated on a seedling tray until 2-4 true leaves appeared (exclude primordial leaves), equal to 11 days after sowing (DAS). Five plants were transplanted to each plastic pot filled with mixed substrates with different concentrations of BSF residue. Nevertheless, the first experiment was not using any added fertilizer other than the BSF residue. Growth data were collected 7-35 days after transplanting (DAT). The amaranth plants were harvested on 21, 28, and 35 DAT.

Table 1. Chemical characteristics of the BSF residue, soil used in this study, and the maximum permissible addition for the selected heavy metal elements

Chemical characteristic	Material		Maximum permissible addition (MPA)	Test method
	Soil	BSF residue		
pH	6.63	6.92		Electrometry
N-total (%)	0.27	1.86		Kjeldahl-titrimetry
P_2O_5-total (mg/100g)	720.53	3548.68		Atomic absorption spectrometry
K_2O-total (mg/100g)	63.94	1674.05		
Mg-total (mg/100g)	123.09	1133.87		
Cu in DTPA (ppm)	4.16	15.58	3.5*	
Zn in DTPA (ppm)	7.73	40.21	16*	
Mn in DTPA (ppm)	6.87	23.10	12**	
Available P Bray II (ppm)	1306.40	13543.04		Spectrophotometry

*Vodyanitskii (2016), **WHO-FAO (1996)

2.3. Experiment 2: Population Density and BSF-Residue Application

The second experiment aimed to optimize the yield of red amaranth using lower BSF residue, optimal population density, and the low-cost conventional cultivation system. The second experiment was redesigned based on the results of the first experiment, which shows a high concentration of BSF residue (30% and 50%) significantly inhibited plant height, the number of leaves, leaf area, fresh and dry weight of leaves, stems, roots, and root length. Therefore, the second experiment lowered the application rates to 20% or lower. Furthermore, population density treatment was added to the second experiment. Last, the cultivation system was changed to the conventional system since the BSF residue was easily dissolved in water and leaked out of the growing media through the drainage holes underneath the pots and side holes during rain.

Experiment 2 adhered to the Randomized Block Design with two factors, i.e., the rates of BSF residue and plant population density. Each block consisted of 5 population densities combined with 3 concentrations of the BSF residue mixed with a soil-based media. Population density was generated by different weights of seeds per pot, i.e., 0.1 mg; 0.2 mg; 0.3 mg; 0.4 mg; and 0.5 mg. Concentrations of the BSF residue were 0%, 10%, and 20%. Each treatment combination was replicated 3 times.

The seeds of the red amaranth were tiny, with a diameter of less than 1 mm. Therefore, the seeds were directly spread on the surface of the growing media; then covered with a thin layer of the same media. No transplanting was done as in Experiment 1. In Experiment 2, NPK 16:16:16 was applied as basic fertilizer at a rate of 2 g per pot before planting and at 1 g of Urea (Nitrogen 46%) per pot for 3 weeks after planting (WAP).

Red amaranth was harvested 25 days after planting (DAP). The harvested plants were categorized as marketable or non-marketable based on height. The marketable plants have heights more than or equal to 15 cm; meanwhile, those shorter than 15 cm were categorized as non-marketable. Amaranth as vegetables is mainly sold, including its roots, but only leaf and stem are consumed.

2.4. Data collection

Data on plant height and the number of leaves were collected every 3 days, starting from 7 to 25 DAT in both Experiment 1 and Experiment 2. SPAD values were measured using a chlorophyll meter (Konica-Minolta SPAD 502 Plus). Leaf area was directly measured using a digital image processing application developed by Easlon and Bloom (2014). However, since the color of amaranth leaves used was red; then, the squared calibration chip was changed to the green color. The reversed calculation was accordingly done for each measured leaf area. Data on other growth and yield traits were measured at harvest, including fresh and dry weights of plant components, i.e., stem, leaf, and roots. Measurements of dry weight were done after each plant component was dried in an oven at 70°C until a steady weight was achieved. The root length was measured from the plant base to the tip of the longest root.

2.5. Statistical Analysis

The analysis of variance was carried out using the SAS 9.0 application for testing the significant effect of each treatment and its interaction with all measured traits. If the treatments significantly affected growth and yield traits, a further test was performed using the Least Significant Difference test at $p<0.05$ ($LSD_{0.05}$) to specify differences amongst levels within each treatment and its interaction.

3. Results

3.1. Effects of BSF Residue on Red Amaranth Growth and Yield

Effects of BSF application on the growth of the red amaranth were not linear. A positive response of red amaranth was observed if the growing substrate was added with 10% BSF on a volume basis (v/v). It was unexpected that the growth of red amaranth was significantly halted at higher BSF application rates, i.e., 30% and 50%. The stunting effects were observed in plant height and leaf number, respectively (Table 2). Results of soil analysis disclosed that the stunting was associated with a high concentration of heavy metal elements, i.e., Cu, Zn, and Mn, as shown in Table 1.

Table 2. Plant height and leaf number during early vegetative growth in red amaranth cultivated on a soil-based substrate treated with different rates of black soldier fly residue

Plant age (day)	Rate of black soldier fly residue (%)							
	0	10	30	50	0	10	30	50
	Plant height (cm)				Number of leaves			
7	2.6±0.8^{a}	2.7±0.9^{a}	2.2±0.4^{a}	1.4±0.2^{b}	2.1±0.4^{a}	2.2±0.6^{a}	2.0±0.5^{a}	1.1±0.3^{b}
10	3.8±0.8^{a}	3.3±1.0^{a}	3.3±0.4^{a}	1.9±0.2^{b}	2.9±0.4^{b}	3.4±0.4^{a}	2.8±0.3^{b}	1.6±0.4^{c}
13	4.0±0.7^{b}	4.7±1.0^{a}	3.3±0.4^{c}	2.5±0.5^{d}	4.0±0.5^{b}	4.7±0.6^{a}	3.6±0.3^{c}	2.6±0.7^{d}
16	5.0±1.0^{b}	5.9±1.3^{a}	4.0±0.6^{c}	2.9±0.6^{d}	4.6±0.4^{b}	5.4±0.5^{a}	4.0±0.4^{c}	3.2±0.8^{d}
19	6.0±0.9^{b}	7.7±1.3^{a}	4.8±0.8^{c}	3.0±0.6^{d}	6.2±0.7^{b}	7.5±0.8^{a}	5.5±0.8^{c}	4.0±0.8^{d}
22	7.0±1.1^{b}	9.3±1.1^{a}	5.1±1.0^{c}	3.1±1.0^{d}	6.7±0.7^{b}	8.0±0.7^{a}	5.8±0.9^{b}	4.1±0.5^{c}
25	8.6±1.0^{b}	10.3±1.4^{a}	5.4±0.9^{c}	4.0±0.7^{c}	8.3±0.5^{b}	9.6±0.5^{a}	5.4±0.5^{c}	5.3±1.3^{c}
28	9.4±1.0^{b}	12.1±1.4^{a}	5.9±1.4^{c}	4.4±1.3^{c}	8.9±0.9^{a}	10.3±0.7^{a}	6.1±1.9^{b}	5.4±1.4^{b}
31	10.6±1.2^{ab}	12.7±2.4^{a}	7.2±2.7^{bc}	3.9±0.7^{c}	9.9±0.7^{ab}	10.9±1.4^{a}	6.0±2.8^{bc}	5.6±1.5^{c}
34	11.8±1.3^{ab}	14.4±3.1^{a}	7.8±2.1^{bc}	3.8±1.1^{c}	10.5±0.2a	11.4±1.2^{a}	5.6±2.1^{b}	4.7±0.7^{b}

Results of experiment 1. Means followed by different letters in each row for each trait were significantly different at $LSD_{0.05}$

Data collected each week were chosen from within each rate of BSF residue treatment. Data on root traits were collected based on the destructive procedure. The BSF treatments exhibited almost consistently significant effects on all measured traits, i.e., fresh and dry weights of the leaf, stem, and roots; leaf area; and length of the longest root. The exceptions were only on leaf dry weight at 3 WAT and root dry weight at 5 WAT (Table 3).

The red amaranth grown on a substrate with 10% BSF residues grow faster than those non-treated or treated with a higher rate of BSF residues. Higher BSF residue treatments (30% and 50%) inhibited the growth of the red amaranth plants (Table 4).

Direct comparison between application rates of the BSF residue at 10% and 20% using the t-test disclosed significant differences between the two rates on consumable components in red amaranth. In this case, consumable and yield components were differentiated. Consumable components cover only leaf and stem fresh weight, while yield components include roots. Nonetheless, it was unanimous that 10% was better than 20% of the BSF application rate in all measured traits (Table 5).

3.2. Effect of the BSF Residue and Plant Density on Growth and Marketable Yield

The application rate of the BSF residue was lowered to 0%, 10%, and 20% in Experiment 2 and combined with seed planting rate treatments. Seed planting rates were applied to create differences in the population density. The BSF treatments significantly affected fresh and dry weights of all plant organs and leaf areas in red amaranth plants; meanwhile, seed planting rate treatments significantly affected

Table 3. Analysis of variance on some growth traits in red amaranth as affected by the black soldier fly residue rate, measured at 3, 4, and 5 weeks after transplanting

Traits	F value		
	3 WAT	4 WAT	5 WAT
Leaf fresh weight	34.81**	33.69**	6.62*
Leaf dry weight	4.45^{ns}	88.25**	6.83*
Stem fresh weight	36.26**	33.27**	5.77*
Stem dry weight	14.25**	45.47**	9.26**
Root fresh weight	16.13**	50.79**	8.16*
Root dry weight	24.28**	34.85**	4.58^{ns}
Root length	19.79**	32.38*	37.14**
Leaf area	12.85**	42.41**	6.50*

Results of experiment 1. * = significant at $F_{0.05}$, ** = significant at $F_{0.01}$, ns = not significant

population density, and total fresh weight yet did not affect the average plant weight (Table 6).

The visual comparison in Figure 1 deceivably indicated that the combination of 10% BSF residue and 50 mg/pot seed planting rates was the best option for the cultivation of red amaranth. However, there was fierce competition amongst plants at a high population density. The canopy of the fast-growing taller plants densely concealed the plants underneath from sunlight. Lack of sunlight caused plants under the canopy to become stunted and non-marketable.

Interpreting collected data on the total number and fresh weight of harvestable plants is straightforward, but it may not be the case for data on average fresh weight per plant in red amaranth. Excessively larger red amaranth plants may not be desirable for consumers. Higher marketable yield was observed at a rate of 30 mg seed per pot or equivalent to 6 mg/dm^2 seeds per substrate surface area (Table 7).

Table 4. Effects of black soldier fly residue on some growth-related parameters at 3 to 5 weeks after red amaranth transplant

BSF residue (%)	LFW (g)	LDW (g)	SFW (g)	SDW (g)	RFW (g)	RDW (g)	RL (cm)	LA (cm^2)
				3 WAT				
0	0.77±0.12^{b}	0.11±0.03	0.32±0.16^{b}	0.031±0.015^{b}	0.44±0.14^{b}	0.036±0.005^{b}	11.78±4.41^{a}	66.76±30.82^{b}
10	1.83±0.44^{a}	0.27±0.17	0.90±0.16^{a}	0.081±0.027^{a}	1.17±0.40^{a}	0.069±0.020^{a}	15.84±1.45^{a}	127.34±30,24^{a}
30	0.14±0.08^{c}	0.03±0.02	0.11±0.04b^{c}	0.019±0.005^{b}	0.09±0.03^{b}	0.009±0.003^{c}	2.52±0.86^{b}	30.24±28.31^{bc}
50	0.08±0.06^{c}	0.01±0.01	0.03±0.03^{c}	0.004±0.005^{b}	0.05±0.04^{b}	0.004±0.002^{c}	2.39±0.62^{b}	10.16±11,03^{c}
LSD 5%	0.48	0.19	0.23	0.031	0.45	0.020	0.526	49.67
				4 WAT				
0	1.45±0.14^{b}	0.20±0.01^{b}	1.21±0.34^{b}	0.11±0.02^{b}	0.82±0.49^{b}	0.12±0.06^{b}	18.81±10.57^{a}	71.15±39.11^{b}
10	2.86±0.23^{a}	0.39±0.03^{a}	2.25±0.24^{a}	0.25±0.08^{a}	2.34±0.28^{a}	0.22±0.02^{a}	19.88±4.75^{a}	129.41±32.41^{a}
30	0.64±0.03^{c}	0.07±0.01^{c}	0.36±0.08^{c}	0.04±0.04^{bc}	0.43±0.04^{b}	0.04±0.01^{c}	4.67±0.92^{b}	16.08±6,05^{c}
50	0.31±0.30^{c}	0.06±0.03^{c}	0.27±0.15^{c}	0.03±0.13^{c}	0.21±0.13^{b}	0.03±0.02^{c}	3.85±1.04^{b}	12.31±6,27^{c}
LSD 5%	0.44	0.04	0.45	0.78	0.65	0.07	11.83	43.77
				5 WAT				
0	2.13±0.40^{ab}	0.32±0.05^{ab}	1.37±0.03^{ab}	0.20±0.05^{b}	2.18±0.87^{ab}	0.23±0.05	17.45±0.31^{a}	68.84±21.19^{ab}
10	3.41±1.73^{a}	0.55±0.23^{a}	2.59±1.17^{a}	0.37±0.10^{a}	3.36±1.37^{a}	0.45±0.29	19.84±2.85^{a}	150.26±88.80^{a}
30	0.50±0.48^{b}	0.11±0.12^{b}	0.48±0.63^{b}	0.09±0.09^{b}	0.43±0.53^{bc}	0.05±0.06	3.57±3.35^{b}	22.00±8.25^{b}
50	0.24±0.12^{b}	0.05±0.03^{b}	0.21±0.16^{b}	0.04±0.28^{b}	0.27±0.28^{b}	0.02±0.01	2.58±0.74^{b}	12.89±23.28^{b}
LSD 5%	1.99	0.04	1.54	0.17	0.65	0.32	5.15	85.25

Results of experiment 1. BSF = black soldier fly, LFW = leaf fresh weight, LDW = leaf dry weight, SFW = stem fresh weight, SDW = stem dry weight, RFW = root fresh weight, RDW = root dry weight, RL = root length, LA = leaf area, LSD = least significant difference, and WAT = weeks after planting. Means followed by different letters in each column at each week of observation are not significantly different

Table 5. Paired comparison between red amaranth treated with 10% and 20% black soldier fly (BSF) residue

Measured trait	BSF rate (%)		Difference		t-value		
	10	20	Mean	SE	Calculated	Table 5	Table 1
Leaf fresh weight (g)	5.053±1.904	3.623±0.848	1.430	0.273	5.244**		
Leaf dry weight (g)	0.565±0.226	0.413±0.131	0.153	0.024	6.241**		
Stem fresh weight (g)	6.940±2.894	4.532±1.311	2.408	0.409	5.891**		
Stem dry weight (g)	0.346±0.098	0.240±0.082	0.107	0.004	26.124**		
Root fresh weight (g)	1.751±0.726	1.067±0.495	0.684	0.060	11.452**	1.761	2.624
Root dry weight (g)	0.131±0.085	0.086±0.042	0.045	0.011	4.100**		
Root length (cm)	15.871±2.914	16.297±2.834	-0.427	0.021	20.579**		
Leaf area (dm^2)	2.896±0.133	1.898±0.483	0.998	0.219	4.552**		

Results of experiment 2. SE = standard error, **Significant difference based on t-test at p<0.01

Table 6. Analysis of variance on some growth traits of red amaranth affected by black soldier fly (BSF) residue rates and seed planting rate

Trait	F-value		
	BSF residue	Seed planting rate	Interaction
Leaf fresh weight	11.39**	2.2^{ns}	2.08^{ns}
Leaf dry weight	7.04*	0.73^{ns}	0.92^{ns}
Stem fresh weight	9.47**	0.32^{ns}	0.77^{ns}
Stem dry weight	22**	1.09^{ns}	1.85^{ns}
Root fresh weight	13.52**	1.32^{ns}	1.23^{ns}
Root dry weight	5.36*	1.9^{ns}	0.78^{ns}
Root length	0.22^{ns}	2.32^{ns}	0.39^{ns}
Number of plants	0.91^{ns}	5.44**	0.52^{ns}
Total weight	3.77^{ns}	5.09**	0.47^{ns}
Average weight	9.80**	1.03^{ns}	0.89^{ns}
Leaf area	9.04**	0.86^{ns}	0.77^{ns}

Results of experiment 2. * = significant at $F_{0.05}$, ** = significant at $F_{0.01}$; ns = not significant

Higher seed planting density increased the number of red amaranth plants per pot; however, higher population density decreased the percentage of marketable-size plants. Without additional nutrients, the growth of red amaranth plants could not reach the marketable size (Table 8). The optimum seed planting density was 30 mg/pot (Table 7) and the optimum application rate of BSF residue was 10 % of soil-based growing substrate (Table 5). The higher population density intensifies competition amongst plants within a limited growing space, causing an increase in non-marketable plants.

3.3. Estimating Leaf Area using Allometric Measurements

The length (L) and width (W) of the leaf blade can be non-destructively measured at any time and

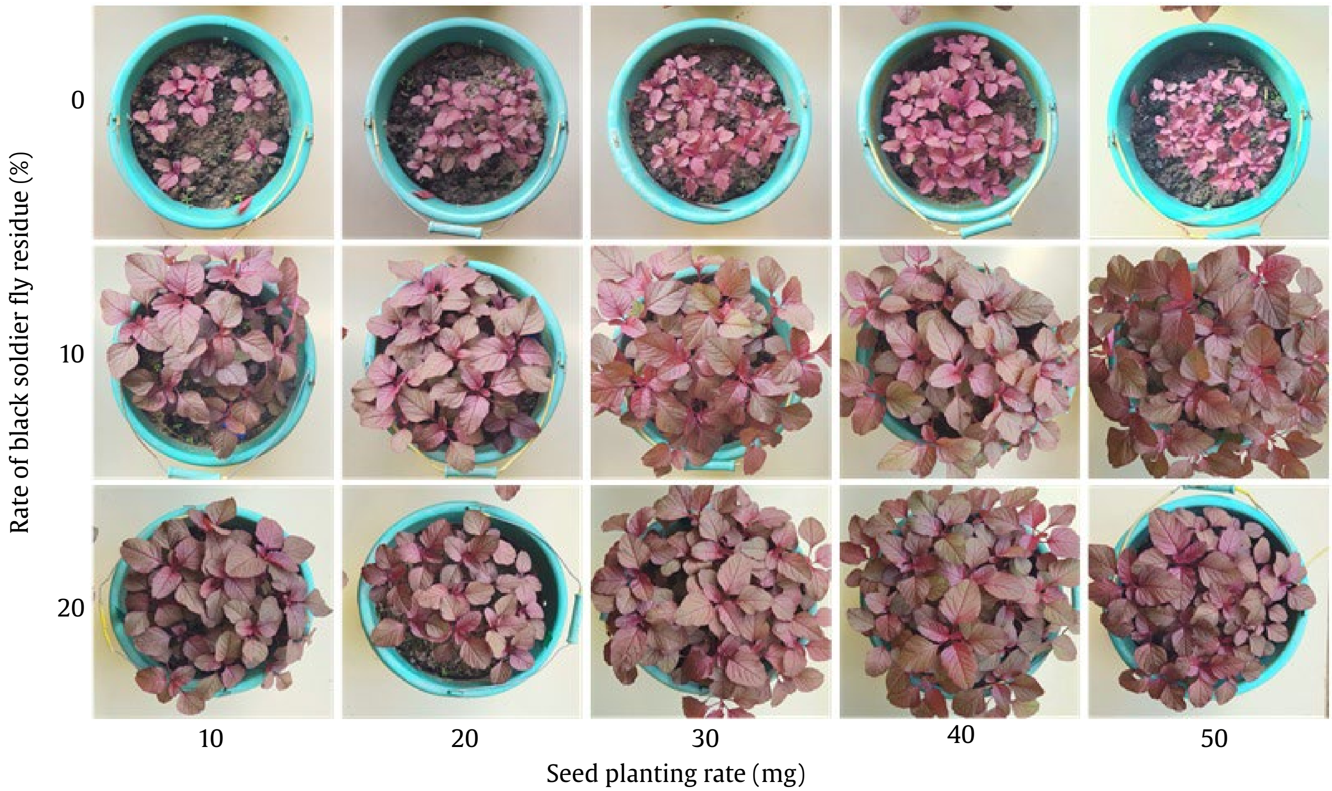

Figure 1. Visual comparison amongst red amaranth grown on soil-based substrate mixed with different BSF residue application rates and seed planting rates (Experiment 2)

Table 7. Effects of seed planting density on total marketable plants, total fresh weight, and average weight per plant

Seed planting density (mg)	Total marketable plant	Total fresh weight (g)	Average weight per plant (g)
10	7.17±3.06^{b}	71.58±26.49^{c}	9.98±6.31^{a}
20	12.17±1.94^{a}	103.25±18.39^{bc}	8.13±2.60^{a}
30	15.17±3.31^{a}	137.00±45.35^{a}	9.03±3.00^{a}
40	13.50±2.17^{a}	122.78±23.82^{ab}	9.09±2.49^{a}
50	13.83±3.97^{a}	115.43±33.94^{ab}	8.35±1.34^{a}
LSD 5%	3.90	32.55	3.28

Results of experiment 2. Means followed by different letters in each column are significantly different at $LSD_{0.05}$

are repeatable. Therefore, these two morphological traits are frequently used as a predictor in leaf area estimation models using quadratic or power regression. The combination of leaf length and width (LW) increases the accuracy of the estimation model. The zero-intercept linear regression model using LW as a predictor created a highly accurate leaf area estimation in red amaranth (Figure 2). The reliability of the zero-intercept linear and quadratic models using LW as a predictor and the power regression model using L or W as a predictor has been validated (Figure 3).

Table 8. Effects of the seed planting density and concentration of the black soldier fly residue on a ratio of marketable/non-marketable yield and total plant harvested in red spinach

Seed weight/pot (mg)	BSF dropping (%)	Ratio marketable/non-marketable yield (%)	Total plants harvested/pot
50	20	36	39
	10	33	44
	0	100	29
40	20	41	39
	10	36	35
	0	100	29
30	20	51	33
	10	53	26
	0	100	34
20	20	48	27
	10	53	22
	0	100	17
10	20	61	11
	10	62	13
	0	100	19

Results of experiment 2. The green bar is for marketable yield, the red bar is for non-marketable yield, and the blue bar is for the total number of plants

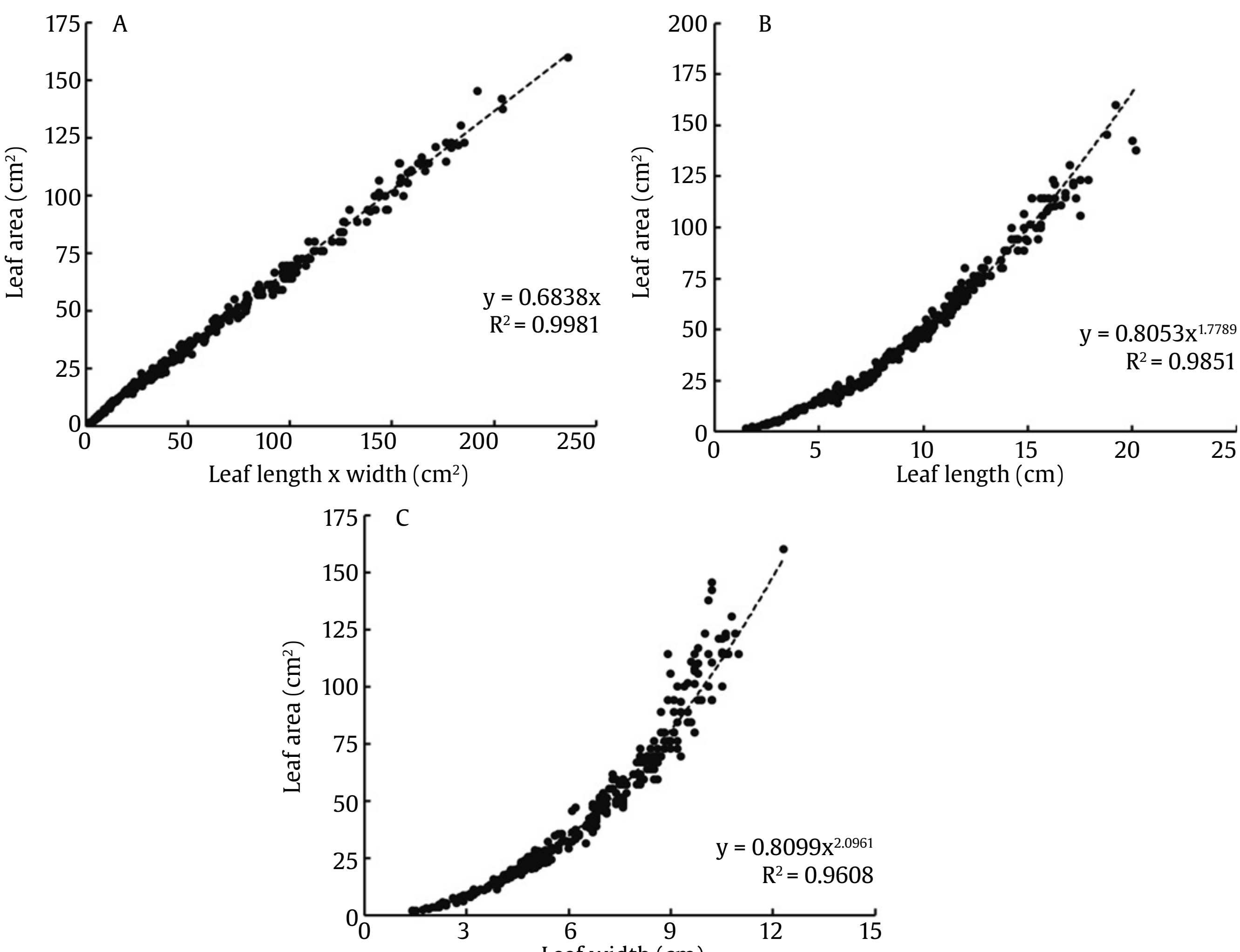

Figure 2. Accuracy of leaf length x width (A), leaf length (B), and leaf width (C) as predictors combined with zero-intercept linear (A) or power (B and C) in estimating leaf area of red amaranth plants (experiment 2)

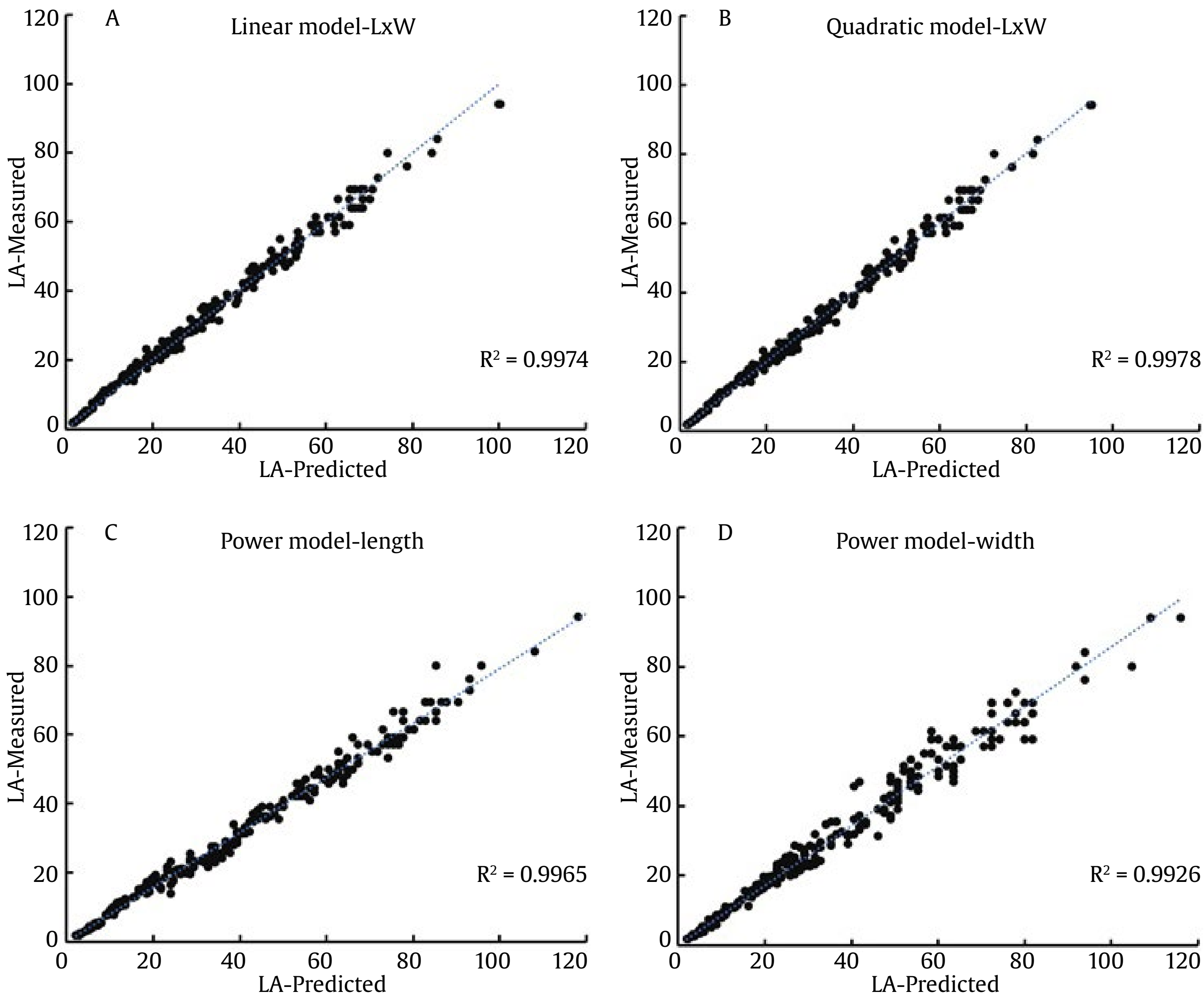

Figure 3. Reliability of leaf length x width as a predictor using zero-intercept linear (A) or quadratic regression (B) and leaf length (C) or width (D) as predictor using power regression in estimating the leaf area in red amaranth (experiment 2)

4. Discussion

4.1. Effects of BSF Residue on Red Amaranth Growth and Yield

The growth and yields of red amaranth performed better on an application rate of 10% BSF residue than control or higher application rates in floating culture or conventional culture systems (Jaya *et al.* 2019, 2021; Kartika *et al.* 2021; Siaga *et al.* 2018). The 10% rate is appropriate for BSF residue because it contains high nutrients (both macro and micro) and high organic matter. Kawasaki *et al.* (2020) reported that residue of household waste processed with BSF contained a high concentration of NH_4^+-N but low NO_3-N. Furthermore, applying this BSF residue significantly increased the above-ground dry weight of komatsuna (*Brassica rapa var. perviridis*). However, the residue may contain pathogenic Xanthomonadaceae bacteria, which should be considered.

The combination of both nitrogen forms increases carbon accumulation in a plant (Wang *et al.* 2019). The carbon accumulation was associated with higher yields in red amaranth. The fresh shoot weight determined the yield quantity of red amaranth, yet younger plant was preferred by consumers (Gil *et al.* 2012). Therefore, the fast-growing type is more suitable for red amaranth production.

It is expected that roots are suffered more since the source of the problem was in the growing substrate. However, the roots were healthy, as indicated by higher root biomass if 10% BSF was mixed into the soil-based substrate, compared to higher BSF application rates, i.e., the root elongation was suppressed by 30-50% at higher application rates (Table 2). In contrast, Borkent and Hodge (2021) reported that the application of BSF waste product increased the dry weight of the shoot and root of several herb and vegetable plants and had similar effects to other commonly-used organic fertilizers, such as chicken manure.

The stunted growth of the amaranth plants was due to the high concentration of heavy metals in the BSF residues. The undersized effects were observed on all plant parts (Table 3). Since the source of the problem was in the growing substrate; then, the first suffering organ was the roots, as indicated by smaller root biomass and shorter roots (Liu *et al.* 2021). The uptake of water and nutrients by affected plants becomes restricted and collectively halts the development of aerial organs, including leaf enlargement (Bellache *et al.* 2022).

Negative effects of an overdose in BSF rates on the plant had been reported in other plants. For instance, the total fresh weight of spinach decreased significantly at a 40% BSF rate (Bortolini *et al.* 2020); growth and yield in rice were reduced at BSF application rates as low as 6 to 8% (Wu *et al.* 2020). Hence, BSF residue in red amaranth should not be applied at a rate higher than 10% (Table 4).

BSF residue at a lower concentration can be beneficial and efficient compared to other organic fertilizers as long as it does not exceed the upper threshold value of 10% for red amaranth (Table 5). Kawasaki *et al.* (2020) calculated that to obtain 100 mg of nitrogen, the required amount of application was 4.75 g for BSF residue; meanwhile, for other fertilizers were 6.44 g, 10.25 g, and 14.72 g for cow manure, composted household organic waste, and horse manure, respectively. Benefits of using BSF residue or at least comparable to inorganic fertilizers as a nutrient source for crops have been reported in many cases, including on lettuce (Bortolini *et al.* 2020), chili pepper (Quilliam *et al.* 2020), and maize (Tanga *et al.* 2021).

Organic waste often contains heavy metals that may accumulate in the larvae and prepupae of the BSF and, consequently, in the food chain. The heavy metals accumulated in the BSF body or the BSF droppings included cadmium, lead, and zinc (Diener *et al.* 2015; Purschke *et al.* 2017). Therefore, using BSF residue as organic fertilizer required monitoring, especially for Cd and Pb, to ensure food safety along the value chain.

Cai *et al.* (2018) revealed that adding chicken manure and wheat bran as co-substrates improved the conversion process, which was influenced by the nature and amount of added co-substrate, especially the quantity of the added nitrogen. With the amended substrate, the heavy metal content of the treatment residue was lower than that considered safe for organic-inorganic compound fertilizers standards (Vodyanitskii 2016). The concentration of heavy metals accumulated in BSF residue was much higher than those of non-contaminated soil.

4.2. Effect of the BSF Residue and Plant Density on Growth and Marketable Yield

The BSF residue affected the growth of the red amaranth plant's leaf, stem, and roots; meanwhile, population density affected marketable yield (Table 6, Figure 1). The number of the marketable plant increased as population density increased up to 30 mg/dm^2 seed per pot, then declined at higher population density. Plant individual size was very diverse at higher population density, i.e., planted with seeds at 40 to 50 mg/dm^2 (Table 7). Postma *et al.* (2021) agreed that increasing density increased the standing crop per area but decreased the individuals' mean size. A similar result was reported by Zhang *et al.* (2020) that the negative effect of population density on growth also occurred on aquatic plants Spirodela polyrhiza, and the impact was more significant under high than under low nutrient availability. Adams *et al.* (2019) reminded us that excessive over-seeding might occur in many cases and result in economic losses to farmers.

Earlier emergence plants grew faster and bigger. Therefore, the late emergence plants were stunted due to low available light under the dense canopy of the earlier emergence plants. Postma *et al.* (2021) concluded, based on their study, that the high population densities correlated strongly with low light intensity due to shading effects more than nutrient depletion. Based on the total fresh weight of the marketable plants, the highest yield was achieved in a combination of 30 mg/dm^2 population density and BSF residue application at 10% in the mixed growing media (Table 8).

4.3. Estimating Leaf Area using Allometric Measurements

The leaves are a vital organ, from the perspective of plant biology, especially in capturing sunlight energy, and agronomy, mainly because the leaves are the yield organs with economic value. The model of leaf area estimation is urgently needed and makes it possible to non-destructively monitor the development of the same leaf during its development. This model of leaf area estimation has been proven to be accurate. Furthermore, the grading of vegetable crops can be easily carried out based on the size of the leaf area.

Length of the midrib (L) and width of the leaf blade (W) as predictors have been proven to be accurate and reliable for estimating the symmetric and regular-shape leaf area (LA) of the red amaranth using a power regression model (Figure 2). Meanwhile, the combination of midrib length and blade width (LW) as predictors increased the accuracy and reliability of the leaf area estimation using the zero-intercept linear regression model (Figure 3). Linear model using LW as a predictor (independent variable) increased accuracy in estimating leaf area than the use of L or W separately in chrysanthemum (Fanourakis *et al.* 2021) and Stevia (Hernández-Fernandéz *et al.* 2021). These approaches have been provenly accurate for single and regular-shape leaves.

There are some challenges in estimating the LA of multiple and irregularly-arranged leaflets, such as the compound leaf of the konjac plant (Amorphophallus muelleri). Nurshanti *et al.* (2022) genuinely developed an accurate LA estimation model (R^2 = 0.9932) for the compound leaf of the konjac plant using the zero-intercept linear model and the multiplication of total length of leaflet midribs (TLM), and an average width of leaflet blades (AWL) was used as a single predictor. The coefficient of determination (R^2) for the width of leaf blade (W) as a predictor and using power regression, midrib length (L) as a predictor and using power regression, and LW as predictor using zero-intercept linear regression were 0.9608, 0.9851, and 0.9981, respectively. All of the LA estimation models had been validated using a different data set.

In conclusion, red amaranth can optimally grow at a seed density of 30 mg/dm^2. Application at higher density causes plant-plant competition, thus creating high size variability within the population or reducing the average size of the plants. Application of BSF residue as a source of plant nutrients should be cautiously made since it may contain some heavy metals at a rate much higher than the required amount for optimum growth. In addition, the leaf area of red amaranth can be accurately estimated using the LW predictor using the zero-intercept regression model.

Acknowledgments

We sincerely appreciated suggestions, comments, and corrections from the editor-in-chief, co-editors, and anonymous reviewers. This research was funded by the Program Penelitian Profesi, Universitas Sriwijaya, Indonesia. SK No. 0111/UN9.3.1/SK/2022.

References

Adams, C., Thapa, S., Kimura, E., 2019. Determination of a plant population density threshold for optimizing cotton lint yield: a synthesis. *Field Crops Research*. 230, 11-16. https://doi.org/10.1016/j.fcr.2018.10.005

Barragan-Fonseca, K.B., Dicke, M., van Loon, J.J., 2017. Nutritional value of the black soldier fly (*Hermetia illucens* L.) and its suitability as animal feed–a review. *Journal of Insects as Food and Feed*. 3, 105-120. https://doi.org/10.3920/JIFF2016.0055

Bellache, M., Allal Benfekih, L., Torres-Pagan, N., Mir, R., Verdeguer, M., Vicente, O., Boscaiu, M., 2022. Effects of four-week exposure to salt treatments on germination and growth of two *Amaranthus* species. *Soil Systems*, 6, 57. https://doi.org/10.3390/soilsystems6030057

Bokau, R.J., Witoko, P., 2017. Optimalization of bioconversion process of palm kernel cake for production maggot *Hermetia Illucens* as a source of animal protein in fish farming. *Aquacultura Indonesiana*. 18, 20. https://doi.org/10.21534/ai.v18i1.41

Borkent, S., Hodge, S., 2021. Glasshouse evaluation of the black soldier fly waste product HexaFrass™ as an organic fertilizer. *Insects*. 12, 977. https://doi.org/10.3390/insects12110977

Bortolini, S., Macavei, L.I., Hadj Saadoun, J., Foca, G., Ulrici, A., Bernini, F., Malferrari, D., Setti, L., Ronga, D., Maistrello, L., 2020. *Hermetia illucens* (L.) larvae as chicken manure management tool for circular economy. *Journal of Cleaner Production*. 262, 121289. https://doi.org/10.1016/j.jclepro.2020.121289

Cai, M., Hu, R., Zhang, K., Ma, S., Zheng, L., Yu, Z., Zhang, J., 2018. Resistance of black soldier fly (Diptera: Stratiomyidae) larvae to combined heavy metals and potential application in municipal sewage sludge treatment. *Environmental Science and Pollution Research*. 25, 1559-1567. https://doi.org/10.1007/s11356-017-0541-x

Chirere, T.E.S., Khalil, S., Lalander, C., 2021. Fertiliser effect on Swiss chard of black soldier fly larvae-frass compost made from waste and faeces. *Journal of Insects as Food and Feed*. 7, 457-469. https://doi.org/10.3920/JIFF2020.0120

Cickova, H., Newton, G. L., Lacy, R.C., Kozánek, M., 2015. The use of fly larvae for organic waste treatment. *Waste Management*. 35, 68–80. https://doi.org/10.1016/j.wasman.2014.09.026

Dickinson, E., Harrison, M., Parker, M., Dickinson, M., Donarski, J., Charlton, A., Nolan, R., Rafat, A., Gschwend, F., Hallett, J., Wakefield, M., Wilson, J., 2019. From waste to food: optimising the breakdown of oil palm waste to provide substrate for insects farmed as animal feed. *PloS ONE*. 14, e0224771. https://doi.org/10.1371/journal.pone.0224771

Diener, S., Zurbrügg, C., Tockner, K., 2015. Bioaccumulation of heavy metals in the black soldier fly, *Hermetia illucens* and effects on its life cycle. *Journal of Insects as Food and Feed*. 1, 261-270. https://doi.org/10.3920/JIFF2015.0030

Easlon, H.M., Bloom, A.J., 2014. Easy Leaf Area: automated digital image analysis for rapid and accurate measurement of leaf area. *Applications in Plant Sciences*. 2, 1400033. https://doi.org/10.3732/apps.1400033

Fanourakis, D., Kazakos, F., Nektarios, P.A., 2021. Allometric individual leaf area estimation in chrysanthemum. *Agronomy*. 11, 795. https://doi.org/10.3390/agronomy11040795

Gengatharan, A., Dykes, G.A., Choo, W.S., 2015. Betalains: natural plant pigments with potential application in functional foods. *LWT-Food Science and Technology*. 64, 645-649. https://doi.org/10.1016/j.lwt.2015.06.052

Gil, M.I., Tudela, J.A., Martínez-Sánchez, A., Luna, M.C., 2012. Harvest maturity indicators of leafy vegetables. *Stewart Postharvest Review*. 8, 1–9. https://doi.org/10.2212/spr.2012.1.2

Hernández-Fernandéz, I.A., Jarma-Orozco, A., Pompelli, M.F., 2021. Allometric models for non-destructive leaf area measurement of stevia: an in depth and complete analysis. *Horticultura Brasileira*. 39, 205-215. https://doi.org/10.1590/s0102-0536-20210212

Jaya, K.K., Lakitan, B., Negara, Z.P., 2019. Depth of water-substrate interface in floating culture and nutrient-enriched substrate effects on green apple eggplant. *Agrivita Journal of Agricultural Science*. 41, 230-237. http://doi.org/10.17503/agrivita.v41i2.2235

Jaya, K.K., Lakitan, B., Bernas, S.M., 2021. Responses of leaf celery to floating culture system with different depths of water-substrate interface and NPK-fertilizer application. *Walailak Journal of Science and Technology*. 18, 19823. https://doi.org/10.48048/wjst.2021.19823

Kartika, K., Lakitan, B., Ria, R.P., Putri, H.H., 2021. Effect of the cultivation systems and split fertilizer applications on the growth and yields of tatsoi (*Brassica rapa* subsp. narinosa). *Trends in Sciences*, 18, 344-344. https://doi.org/10.48048/tis.2021.344

Kawasaki, K., Kawasaki, T., Hirayasu, H., Matsumoto, Y., Fujitani, Y., 2020. Evaluation of fertilizer value of residues obtained after processing household organic waste with black soldier fly larvae (*Hermetia illucens*). *Sustainability*. 12, 4920. https://doi.org/10.3390/SU12124920

Lakitan, B., Hadi, B., Herlinda, S., Siaga, E., Widuri, L.I., Kartika, K., Meihana, M., 2018. Recognizing farmers' practices and constraints for intensifying rice production at Riparian Wetlands in Indonesia. *NJAS-Wageningen Journal of Life Sciences*. 85, 10-20. https://doi.org/10.1016/j.njas.2018.05.004

Lakitan, B., Lindiana, L., Widuri, L.I., Kartika, K., Siaga, E., Meihana, M., Wijaya, A., 2019. Inclusive and ecologically-sound food crop cultivation at tropical non-tidal wetlands in Indonesia. *AGRIVITA, Journal of Agricultural Science*, 41, 23-31. http://doi.org/10.17503/agrivita.v40i0.1717

Liu, C., Xiao, R., Dai, W., Huang, F., Yang, X., 2021. Cadmium accumulation and physiological response of *Amaranthus tricolor* L. under soil and atmospheric stresses. *Environmental Science and Pollution Research*. 28, 14041-14053. https://doi.org/10.1007/s11356-020-11569-3

Nurshanti, D.F., Lakitan, B., Hasmeda, M., Ferlinahayati, F., Negara, Z.P., Susilawati, S., Budianta, D., 2022. Planting materials, shading effects, and non-destructive estimation of compound leaf area in konjac (*Amorphophallus muelleri*). *Trends in Sciences*. 19, 3973-3973. https://doi.org/10.48048/tis.2022.3973

Onyango, C.M., Harbinson, J., Imungi, J.K., Shibairo, S.S., van Kooten, O., 2012. Influence of organic and mineral fertilization on germination, leaf nitrogen, nitrate accumulation and yield of vegetable amaranth. *Journal of Plant Nutrition*. 35, 342-365. https://doi.org/10.1080/01904167.2012.639917

Postma, J.A., Hecht, V.L., Hikosaka, K., Nord, E.A., Pons, T.L., Poorter, H., 2021. Dividing the pie: a quantitative review on plant density responses. *Plant, Cell and Environment*. 44, 1072-1094. https://doi.org/10.1111/pce.13968

Purschke, B., Scheibelberger, R., Axmann, S., Adler, A., Jäger, H., 2017. Impact of substrate contamination with mycotoxins, heavy metals and pesticides on the growth performance and composition of black soldier fly larvae (*Hermetia illucens*) for use in the feed and food value chain. *Food Additives and Contaminants: Part A*. 34, 1410-1420. https://doi.org/10.1080/19440049.2017.1299946

Quilliam, R.S., Nuku-Adeku, C., Maquart, P., Little, D., Newton, R., Murray, F., 2020. Integrating insect frass biofertilisers into sustainable peri-urban agro-food systems. *Journal of Insects as Food and Feed*. 6, 315–322. https://doi.org/10.3920/JIFF2019.0049

Sarker, U., Oba, S., 2019. Protein, dietary fiber, minerals, antioxidant pigments and phytochemicals, and antioxidant activity in selected red morph *Amaranthus leafy* vegetable. *PLoS ONE*. 14, 1–16. https://doi.org/10.1371/journal.pone.0222517

Siaga, E., Lakitan, B., Bernas, S. M., Wijaya, A., Lisda, R., Ramadhani, F., Widuri, L.I., Kartika, K., Meihana, M., 2018. Application of floating culture system in chili pepper (*Capsicum annum* L.) during prolonged flooding period at riparian wetland in Indonesia. *Australian Journal of Crop Science*. 12, 808-816. https://doi.org/10.21475/ajcs.18.12.05.PNE1007

Siaga, E., Lakitan, B., Hasbi, H., Bernas, S.M., Widuri, L.I., Kartika, K., 2019. Floating seedbed for preparing rice seedlings under unpredictable flooding occurrence at tropical riparian wetland. *Bulgarian Journal of Agricultural Science*. 25, 326-336.

Tanga, C.M., Beesigamukama, D., Kassie, M., Egonyu, P.J., Ghemoh, C.J., Nkoba, K., Subramanian, S., Anyega, A.O., Ekesi, S., 2021. Performance of black soldier fly frass fertiliser on maize (*Zea mays* L.) growth, yield, nutritional quality, and economic returns. *Journal of Insects as Food and Feed*. 1–12. https://doi.org/10.3920/jiff2021.0012

Vodyanitskii, Y.N., 2016. Standards for the contents of heavy metals in soils of some states. *Annals of Agrarian Science*. 14, 257-263. https://doi.org/10.1016/j.aasci.2016.08.011

Wang, P., Wang, Z., Kui, Sun, X., Chao, MU, X., Huan, Chen, H., Chen, F., Jun, Lixing, Y., Mi, G., Hua, 2019. Interaction effect of nitrogen form and planting density on plant growth and nutrient uptake in maize seedlings. *Journal of Integrative Agriculture*. 18, 1120–1129. https://doi.org/10.1016/S2095-3119(18)61977-X

[WHO-FAO] World Health Organization-Food and Agriculture Organization, 1996. *Guidelines for Drinking Water Quality. Vol. 2, Health Criteria and other Supporting Information*, second ed. World Health Organization, Geneva.

Wu, N., Wang, X., Xu, X., Cai, R., Xie, S., 2020. Effects of heavy metals on the bioaccumulation, excretion and gut microbiome of black soldier fly larvae (*Hermetia illucens*). *Ecotoxicology and Environmental Safety*. 192, 110323. https://doi.org/10.1016/j.ecoenv.2020.110323

Zhang, L.M., Jin, Y., Yao, S.M., Lei, N.F., Chen, J.S., Zhang, Q., Yu, F.H., 2020. Growth and morphological responses of duckweed to clonal fragmentation, nutrient availability, and population density. *Frontiers in Plant Science*. 11, 618. https://doi.org/10.3389/fpls.2020.00618

Zhu, F.X., Yao, Y.L., Wang, S.J., Du, R.G., Wang, W.P., Chen, X.Y., Hong, C.L., Qi, B., Xue, Z.Y., Yang, H.Q., 2015. Housefly maggot-treated composting as sustainable option for pig manure management. *Waste Management*. 35, 62–67. https://doi.org/10.1016/j.wasman.2014.10.005