Research Article

Enhancing the Quality of *Kasgot* Biofertilizer Using *Pichia cecembensis* UNJCC Y-157 and Amino Acids to Boost Mustard Greens (*Brassica juncea* L.) Growth

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ABSTRACT

Mustard greens (*Brassica juncea* L.) is a crop with relatively high productivity due to its abundant soil nutritional content. Soil fertility enhancement is commonly achieved through fertilization. *Kasgot* fertilizer, a biofertilizer derived from the residue of black soldier fly (BSF, *Hermetia illucens*) larvae that consume fermented feed, serves as an eco-friendly option beneficial for plant growth. This organic waste is fermented with microorganisms, specifically the yeast *Pichia cecembensis* UNJCC Y-157, provided by the Jakarta State University, along with added amino acids. This study aimed to determine the effects of *P. cecembensis* UNJCC Y-157 yeast and amino acids on BSF larval growth and to assess the impact of the resulting *kasgot* fertilizer on mustard greens growth. This research used a completely randomized design (CRD) with three treatments, including one control, with five replications on fermented BSF larvae feed. For the application of *kasgot* fertilizer on mustard greens, four treatments with four replications were applied, with data analyzed using one-way analysis of variance (ANOVA). The results indicated that BSF larvae fed with added *P. cecembensis* UNJCC Y-157 yeast and amino acids had superior wet weight, observed through morphological characteristics. Additionally, in terms of mustard greens growth, parameters such as plant height, leaf count, leaf width, leaf length, and fresh weight showed that *kasgot* fertilizer containing *P. cecembensis* UNJCC Y-157 yeast yielded better than morphological outcomes in the plants.

Keywords: *Pichia cecembensis*, fermented feed, black soldier fly, *Brassica juncea* L., amino acids

Introduction

Mustard greens (*Brassica juncea* L.) is a popular vegetable crop in Indonesia, known for its high nutritional content, including vitamins and minerals. It contains vitamins K, A, C, E, and folate, along with alkaloids, flavonoids, saponins, the amino acid tryptophan, and dietary fiber (Hartati et al., 2022). According to the Statistics Indonesia (2018), mustard greens production in South Sumatra steadily increased in 2015, 2016, and 2017, with yields of 3,246, 3,278, and 3,780 tons, respectively. However, production declined to 3,615 tons in 2018. Factors contributing to this decrease include insufficient fertilization practices.

One approach to improving mustard greens production is through effective fertilization. Mubarok (2019) notes that improper planting techniques and declining soil fertility can negatively impact mustard greens yields. According to Baharudin et al. (2013), organic fertilizers could improve soil biological, chemical, and physical properties, enhancing soil structure. Dahlianah & Novianti (2020) added that continuous, high use of inorganic fertilizers had adverse effects, leading to soil degradation as organic matter diminishes. Eco-friendly biological agents and organic fertilizers can also help reduce organic waste in the environment. Maggots, specifically, can serve as biodegraders, capable of processing organic waste at a rate of 2–5 times their body weight within 24 h (Wahyuni et al., 2024).

The by-products of maggot processing retain biomass that can be utilized in agriculture as organic fertilizer. Residual material from black soldier fly (BSF, *Hermetia illucens*) larvae offers high nutrient levels suitable for use as fertilizer and soil amendments. Studies have shown that *kasgot*, BSF larval residue, contains essential nutrients, including nitrogen (N), phosphorus (P), and potassium (K), which make it an effective organic fertilizer (Erlangga, 2023; Puspitasari et al., 2024). Research indicates that *kasgot* can promote optimal growth in mustard greens at a concentration of 40 g in a vertical cultivation system (Muhadat, 2021). This residue contains essential macro and micronutrients necessary for plant growth, including N (3.276%), P (3.387%), K (9.74%), organic carbon (40.95%), a C/N ratio of 12.50%, and moisture content of 11.04% (Ambarningrum, 2020). Application of *kasgot* organic fertilizer has shown to improve growth in long beans compared to plants grown without fertilizer (Fahmi, 2018).

Kasgot is the residual product of bioconversion performed by BSF larvae, which processes organic waste with the aid of microorganisms. Organic substrates rich in lignocellulose are challenging for larvae to digest, as they lack the enzymes required for lignin breakdown in their gut (Permana et al., 2022). To address this, organic waste is fermented before being used as maggot feed, making it easier for larvae to decompose. During fermentation, various microorganisms play roles, including yeast.

This study focused on mustard greens and aimed to assess the effects of *P. cecembensis* UNJCC Y-157 yeast and amino acids on maggot growth and to evaluate the impact of the resulting *kasgot* fertilizer on mustard greens growth.

Materials and Methods

This study was conducted at the field trial of Innovation Centre of Tropical Science (ICTS) in Bogor over approximately 4 months, from July to November 2023. The equipment used included test tubes, inoculating needles, containers, beakers, Erlenmeyer flasks, volumetric flasks, a digital scale, pipettes, a shaker, a blender, an autoclave, a laminar air flow cabinet, a pH meter, a thermometer, duct tape, polybags, a hoe, a spade, and a measuring tape. The materials included yeast malt extract agar (YMEA), the yeast isolate *P. cecembensis* UNJCC Y-157, yeast malt broth (YMB), BSF eggs, white mustard greens, cabbage, papaya, soybean pulp, 70% alcohol, granulated sugar, pineapple, rice bran, amino acids, distilled water, mustard greens seeds, manure, and rice husks.

An experimental method employed completely randomized design (CRD). The study comprised of three treatments, including one control, with five replications on fermented BSF larvae feed, as shown in Table 1. For the application of *kasgot* fertilizer on mustard greens, four treatments with four replications were conducted, as shown in Table 2. The study involved independent and dependent variables, with *P. cecembensis* UNJCC Y-157 yeast as the independent variable and the organic plant as the dependent variable.

Rejuvenation of P. cecembensis UNJCC Y-157 Yeast Isolate

The study was initially conducted by rejuvenating the *P. cecembensis* UNJCC Y-157 yeast isolate, hatching BSF maggot eggs, preparing *P. cecembensis* UNJCC Y-157 yeast cell suspensions, fermenting the feed, harvesting maggots and frass, and planting mustard greens seeds with the addition of *kasgot* fertilizer. The following describes the research stages.

Table 1. Composition of study to test the effect of *Pichia cecembensis* UNJCC Y-157 yeast addition in fermented feed on BSF larvae weight.

Treatment	Replication	Treatment code
PC (control)	1	PA. 1
	2	PA. 2
	3	PA. 3
	4	PA. 4
	5	PA. 5
PA	1	PB. 1
	2	PB. 2
	3	PB. 3
	4	PB. 4
	5	PB. 5
PB	1	PC. 1
	2	PC. 2
	3	PC. 3
	4	PC. 4
	5	PC. 5

PC (control) = yeast malt broth (YMB) medium + pineapple extract, PA = *P. cecembensis* UNJCC Y-157, PB = *P. cecembensis* UNJCC Y-157 + amino acids.

Yeast isolate rejuvenation followed the procedure of Maya and Alami (2019) by inoculating the yeast culture into YMEA medium using an inoculation needle once. The inoculum was then incubated at room temperature for 48 h. Successful rejuvenation was indicated by yeast growth on the YMA medium. The rejuvenated yeast was stored in a refrigerator for future use. Rejuvenation reactivated the isolate that had been stored in test tubes for a prolonged period.

Hatching BSF Eggs

BSF egg hatching was done using rice bran, following Mokolensang et al. (2018). The bran was mixed with amino acids at a 1:1 ratio, using 400 g of bran and 400 ml of diluted amino acids in a container. BSF eggs (0.5 g) were placed on a mesh above the substrate. The hatching period lasted 2 days, and the larvae were fed at 10 days old.

Preparation of P. cecembensis UNJCC Y-157 Yeast Fermentation Starter

The fermentation starter was prepared based on Putri (2020) by first creating a yeast cell suspension. Grown yeast isolates were added to 10 ml of sterile distilled water and homogenized at 1,000 rpm for 30 sec using a vortex mixer. The suspension was poured into YMB medium with 10% sterile pineapple extract and homogenized again using a rotary shaker at 35 rpm for 48 h. After 48 h, the suspension was added to the fermentation starter containing sterile distilled water, pineapple extract, and sugar and incubated at room temperature for another 48 h.

Table 2. Research design for testing the effect of *kasgot* fertilizer with the addition of *Pichia cecembensis* UNJCC Y-157 yeast in fermented BSF larvae feed on the growth of mustard greens.

Treatment	Replication	Treatment code
Control	1	K.1
	2	K.2
	3	K.3
	4	K.4
PC	1	PA.1
	2	PA.2
	3	PA.3
	4	PA.4
PA	1	PB.1
	2	PB.2
	3	PB.3
	4	PB.4
PB	1	PC.1
	2	PC.2
	3	PC.3
	4	PC.4

Control = soil + rice husk + manure, PC = control *kasgot* fertilizer, PA = *kasgot* fertilizer with *P. cecembensis* UNJCC Y-157. PB = *kasgot* fertilizer with *P. cecembensis* UNJCC Y-157 + amino acids.

Feed Fermentation

Fermentation was conducted using organic waste, including mustard greens, cabbage, papaya, and soybean pulp, following Bay et al. (2022). Each material was weighed to 3.125 kg, with a total of 12.5 kg of feed for each treatment. Treatment A received yeast grown in YMB with 10% pineapple extract, Treatment B received yeast + amino acids, and Treatment C (control) received only YMB

+ 10% pineapple extract. The mixture was covered and left to ferment for 3 days. After fermentation, the feed was filtered and weighed to 2 kg for feeding to 10-day-old BSF larvae. If the feed was too moist, bran was added to prevent larvae from escaping the container.

Harvesting BSF Larvae and Frass

Maggots were harvested after three feeding cycles at 19 days of age. Harvesting involved separating the maggots from the residue they produced (Rodli & Hanim, 2022). The frass was dried under sunlight. The growth of BSF larvae was measured, including wet and dry weights of both maggots and frass.

Planting Mustard Greens Seeds with Kasgot Fertilizer

The experiment was conducted on four 2 m × 0.8 m plots. The soil was loosened with a fork and hoe, then divided into 50 cm × 20 cm plots. Mustard greens seeds were sown in small polybags filled with a soil-frass mixture for each treatment. A control was also included for comparison. The seeds were sown in a nursery for 2 weeks, after which seedlings were transplanted with the addition of *kasgot* fertilizer at 9 g per planting hole and watered every morning and evening. Mustard greens was harvested 23–30 days after sowing by carefully pulling them to avoid breaking the stems (Dharmawan, 2023).

Data Collection Technique

The collected data included maggot weight at harvest, temperature, and pH during feed fermentation for each treatment over 3 days, wet and dry weights of maggot frass, and quantitative growth data of the mustard greens, including plant height, leaf count, leaf length, and leaf width, measured weekly over 3 weeks. Plant wet weight was measured in the third week. The data were statistically analyzed using IBM SPSS Statistics version 25 with one-way analysis of variance (ANOVA) to determine the effect of yeast application on *kasgot* fertilizer for mustard greens growth. If significant effects were found, Duncan's test was conducted at a 5% significance level (α = 5%) to identify significant differences between treatments.

Results and Discussion

Statistical analysis indicates that the treatment involving the addition of *P. cecembensis* UNJCC Y-157 yeast and amino acids (Treatment PC) had a significant effect on BSF larvae wet weight at a 5% significance level. Meanwhile, the treatment with only *P. cecembensis* UNJCC Y-157 yeast showed the highest impact on the growth of mustard greens in terms of seed viability, plant height, leaf count, leaf length, leaf width, and post-harvest plant wet weight.

Table 3. Effect of *Pichia cecembensis* UNJCC Y-157 yeast addition in fermented feed on BSF larvae weight.

Treatment	Wet weight* (g)
PC	505.8^{a}
PA	709.2^{b}
PB	789.0^{b}

PC (control) = yeast malt broth (YMB) medium + pineapple extract, PA = *P. cecembensis* UNJCC Y-157, PB = *P. cecembensis* UNJCC Y-157 + amino acids.
*Duncan's further test results at a 5% significance level are indicated with superscript letters in each column.

This study result indicates that the combination of microorganisms (yeast) and amino acids could enhance feed conversion efficiency in BSF larvae, resulting in better wet weight growth. In Table 3, treatments with *P. cecembensis* UNJCC Y-157 yeast (PA and PB treatments) showed significantly higher wet weights compared to the control (PC), which included amino acids only, without yeast supplementation.

The application of *P. cecembensis* UNJCC Y-157 yeast on mustard greens also yielded positive results, with improvements in various growth aspects, such as seed viability, plant height, leaf count, leaf length and width, and post-harvest plant wet weight. This is likely due to the yeast's ability to supply essential nutrients or facilitate nutrient absorption in plants (Sudirman & Wati, 2020). *P.*

cecembensis yeast is known to contain enzymes that aid in the decomposition of organic compounds, allowing for more efficient nutrient uptake by plants (Wardah, 2024).

Duncan's post hoc analysis at a 5% significance level showed that treatments PB and PA had different superscript letters, indicating a significant difference between treatments in terms of BSF larvae wet weight. According to the table, PB treatment yielded the highest result (789 g), followed by PA (709.2 g), while PC showed the lowest result (505.8 g). This data supports the hypothesis that the addition of *P. cecembensis* UNJCC Y-157 yeast could enhance BSF larval growth and wet weight.

Table 4. Effect of *Pichia cecembensis* UNJCC Y-157 yeast addition in fermented BSF larvae feed on wet and dry weights of maggot frass.

Treatment	Average maggot frass weight*	
	Wet weight (kg)	Dry weight (kg)
PC	3,157[b]	858[b]
PB	2,118[a]	743[a]
PA	2,152[a]	753[a]

PC (control) = yeast malt broth (YMB) medium + pineapple extract, PA = *P. cecembensis* UNJCC Y-157, PB = *P. cecembensis* UNJCC Y-157 + amino acids,
*Duncan's further test results at a 5% significance level are indicated with superscript letters in each column.

Table 5. Effect of *Pichia cecembensis* UNJCC Y-157 yeast addition in fermented BSF larvae feed on viability of mustard greens seeds.

Treatment	Number of germinated seeds	Parameter (%)
Control	55	85
PA	60	94
PB	58	90
PC	58	90

Control = soil + rice husk + manure, PC = control *kasgot* fertilizer, PA = *kasgot* fertilizer with *P. cecembensis* UNJCC Y-157. PB = *kasgot* fertilizer with *P. cecembensis* UNJCC Y-157 + amino acids.

From the measurements of mustard greens height taken weekly after planting (WAP), as presented in Table 8, it is evident that different treatments resulted in varying plant heights. The average leaf width showed a significant increase in treatments supplemented with specific microorganisms and nutrients compared to the control, as indicated by Duncan's post hoc test at a 5% significance level (Table 8). In the control treatment, the average leaf width gradually increased from 3.80 cm at 1 WAP, to 4.76 cm at 2 WAP, and to 5.75 cm at 3 WAP. Plants in this control treatment exhibited lower growth compared to treated samples, likely due to limited nutrient availability (Juwita & Suryadi, 2021).

Table 6. Effect of *Pichia cecembensis* UNJCC Y-157 yeast addition in fermented BSF larvae feed on average height of mustard greens.

Treatment	Average plant height* (cm)		
	1 WAP	2 WAP	3 WAP
Control	8.41[a]	18.00[a]	27.81[a]
PC	10.65[b]	20.00[b]	30.06[b]
PB	11.53[b]	22.65[c]	31.62[c]
PA	13.34[c]	24.96[d]	34.93[d]

WAP = week after planting.
*Duncan's further test results at the 5% significance level are stated indicated with superscript letters in each column.

Table 7. Effect of *Pichia cecembensis* UNJCC Y-157 yeast addition in fermented BSF larvae feed on average number of mustard greens leaves.

Treatment	Number of leaves		
	1 WAP	2 WAP	3 WAP
Kontrol	3.00[a]	5.56[a]	7.50[a]
PC	4.00[b]	7.50[b]	8.56[b]
PB	5.00[c]	8.56[c]	9.87[c]
PA	6.75[d]	9.87[d]	11.87[d]

WAP = week after planting.
*Duncan's further test results at the 5% significance level are stated indicated with superscript letters in each column.

The combination of *P. cecembensis* yeast, amino acids, and nutrients showed a significant impact on increasing mustard greens height (Tables 7–8). Plants receiving PA treatment produced the best results in terms of average leaf width at each WAP. In contrast, the control treatment without any supplementation showed the lowest growth. These results support the hypothesis that the addition of yeast and amino acids could improve nutrient absorption in plants, thereby

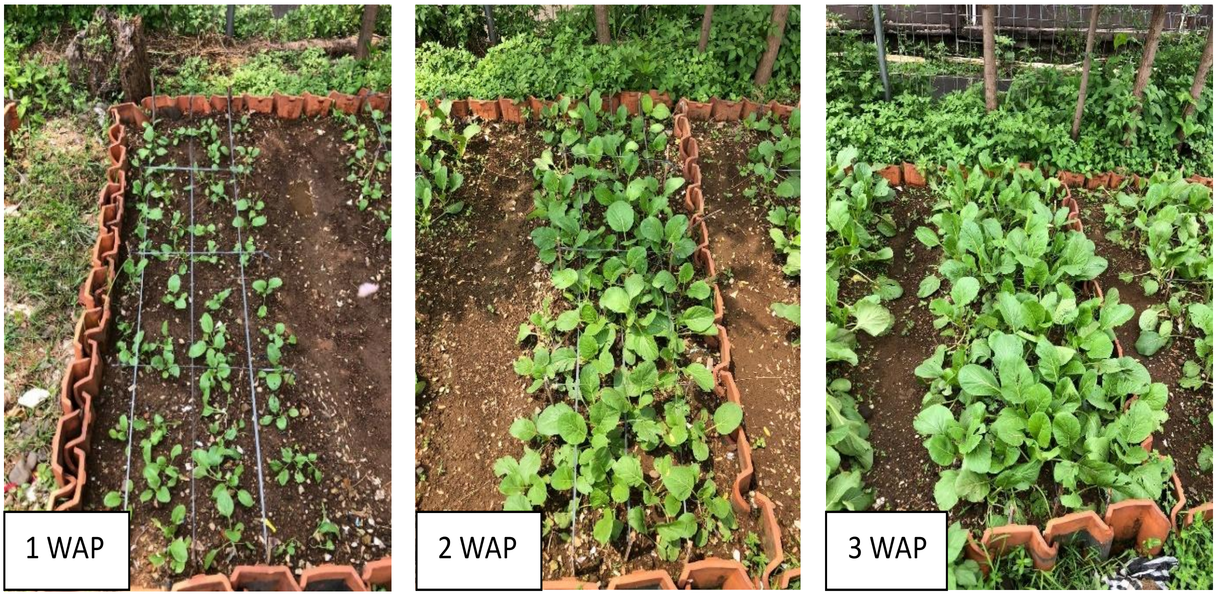

Fig. 1. Effect of *Pichia cecembensis* UNJCC Y-157 yeast addition in fermented BSF larvae feed on growth of mustard greens.

contributing to overall enhanced growth of mustard greens.

Treatment of PB showed the highest wet weight for maggots, reaching 789 g (Table 3). This outcome might be attributed to the addition of *P. cecembensis* UNJCC Y-157 yeast and amino acids. The addition of the amino acid supplement *Agriminovit* has been shown to increase weight in livestock (Jamko, 2013). According to Cafe and Waldroup (2006), the body weight of chickens was influenced by the availability and balance of amino acids in their feed, with methionine and lysine playing crucial roles in tissue growth (Pesti et al., 2005). Additionally, maggot weight is influenced by high-quality feed and the fermentation process. Mustard green waste feed has a relatively high protein content, around 26.33% (Londok et al., 2017). Studies by Tomberlin et al. (2002) and Gobbi et al. (2013) indicate that the quality and quantity of feed consumed by *H. illucens* maggots have significant effects on maggot growth, development time, survival, and mortality rates. The fermentation process can further improve the nutritional quality of the feed. According to Ayuningtyas et al. (2022), microbes or fermentation in growth media could alter the nutritional properties of feed, promoting *H. illucens* larvae growth when the hatching medium has a high protein content.

Table 8. Effect of *Pichia cecembensis* UNJCC Y-157 yeast addition in fermented BSF larvae feed on average leaf width of mustard greens.

Treatment	Average leaf width* (cm)		
	1 WAP	2 WAP	3 WAP
Control	3.80^a	4.76^a	5.75^a
PC	4.08^b	5.48^b	7.21^b
PB	4.16^b	5.78^b	8.68^c
PA	4.75^c	9.00^c	12.43^d

WAP = week after planting.
*Duncan's further test results at the 5% significance level are stated indicated with superscript letters in each column.

Table 9. Effect of *Pichia cecembensis* UNJCC Y-157 yeast addition in fermented feed on average leaf length of mustard greens.

Treatment	Average leaf length* (cm)		
	1 WAP	2 WAP	3 WAP
Kontrol	4.12^a	8.81^a	10.68^a
PC	5.18^b	10.37^b	12.62^b
PB	6.00^c	11.18^c	16.50^c
PA	6.81^d	13.18^d	17.31^d

WAP = week after planting.
*Duncan's further test results at the 5% significance level are stated indicated with superscript letters in each column.

Table 10. Effect of *Pichia cecembensis* UNJCC Y-157 yeast addition in fermented feed on average wet weight of mustard greens harvest after 3 weeks.

Treatment	Wet weight of harvest* (g)
Control	300[a]
PC	475[b]
PB	500[b]
PA	762[c]

*Duncan's further test results at the 5% significance level are stated indicated with superscript letters in each column.

Fig. 2. *Kasgot* fertilizer in three different treatments. PC = control, PA = *Pichia cecembensis* UNJCC Y-157, PB = *P. cecembensis* UNJCC Y-157 + amino acid.

As maggot weight increased, frass production decreased, as seen in Table 4. PA and PB treatments showed reduced frass weight due to the fermentation process with yeast microorganisms. Fermentation broke down the larval feed, making it easier for the maggots to consume. In contrast, PC treatment, which lacked microbial assistance, resulted in slower feed decomposition, requiring the maggots to take longer to consume. Consequently, PC treatment produced more frass than PA and PB treatments. The frass from maggot residues could be used as a plant fertilizer, commonly known as *kasgot* fertilizer. In this study, *kasgot* fertilizer demonstrated beneficial effects on the growth of mustard greens, such as seed viability, plant height, leaf count, leaf length, leaf area, and post-harvest plant wet weight. This is likely because *kasgot* serves as a high-quality organic fertilizer with ample nutrients. Hernahadini (2022) found that BSF maggot residues could be used as an organic fertilizer due to their content of N, P, and K.

The results suggest that nutrients required by plants are present in the *kasgot* fertilizer used in this study. According to the literature, the nitrogen content in *kasgot* fertilizer is relatively high at 2.16%, compared to manure, which has only 0.70% (Sinuraya & Melati, 2019). Nitrogen plays an essential role in promoting vegetative growth, such as increasing leaf count, while phosphorus and potassium contribute to leaf expansion. An increase in leaf count also enhances photosynthesis, leading to greater photosynthate production and more available energy, thereby increasing the plant's final weight (Ambarningrum, 2020).

Conclusion

The addition of *P. cecembensis* UNJCC Y-157 yeast and amino acids significantly influenced the wet weight of BSF larvae. Meanwhile, the application of *kasgot* fertilizer containing *P. cecembensis* UNJCC Y-157 yeast demonstrated superior results compared to the other three treatments. The application of *kasgot* fertilizer on mustard greens yielded superior morphological outcomes, reflecting improved plant growth and appearance.

Conflict of Interest

All authors have no conflicts of interest to disclose.

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