

Research Article

Total glucosinolate content of arugula (*Eruca sativa* Mill.) supplemented with rhizobacteria-enriched bio-slurry

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ABSTRACT

*Arugula is a member of Brassicaceae that has a high antioxidant content of glucosinolate. Bio-slurry is a kind of liquid fertilizer derived from sap of cow dung. Bio-slurry in combination with rhizobacterial can maximize decomposition and make nutrients more available. The research aimed to determine the total glucosinolate content in arugula due to the application of bio-slurry enriched with rhizobacteria. The study used a randomized complete block design with a single factor consisting of 9 levels, i.e., the combination of 3 types of rhizobacteria (*Pseudomonas*, *Bacillus*, *Pseudomonas* + *Bacillus*) and 3 doses of bio-slurry (0, 100, and 200 mL). The results showed that the application of *Pseudomonas* & *Bacillus* + 200 mL bio-slurry produced a higher antioxidant content than other inputs. The combination of bio-slurry fertilizer with rhizobacterial provides a higher ability than control to increase plant growth rates and the biosynthesis of glucosinolate. The optimal substitution for maximizing nutrient uptake in arugula growth was achieved with a bio-slurry dose of 200 mL, where the combined application of *Pseudomonas* and *Bacillus* strains enhanced plant growth and glucosinolate content.*

Keywords: antioxidant capacity; *Bacillus*; bacterial; biofertilizer; *Pseudomonas*

INTRODUCTION

Arugula (*Eruca sativa* Mill.) is a member of Brassicaceae. This plant can be cultivated easily and has the potential as a nutritious microgreen plant. According to Abouelenein et al. (2021), leaf arugula has the most economic value, its slightly bitter and spicy tastes come from glucosinolate compounds which play an important role in suppressing the growth of cancer cells. Glucosinolate compound is a functional antioxidant candidate to combat free radicals. Regular consumption of arugula leaves can help reduce the risk of several serious diseases such as diabetes, cancer, high cholesterol, and obesity (Shubha et al., 2019). According to Rameeh (2015), the glucosinolate content in arugula is higher than other Brassicaceae species; 100 g of fresh arugula leaves contains 140 mg glucosinolate while the others contain about 101 mg.

The quality and phytochemical contents in plants are influenced by agronomic management. Efforts to improve the quality of plants can be made by providing appropriate fertilizers. Providing proper nutrients containing N can support the optimum metabolic process in plants (Yafizham, 2016). Using fertilizer from bio-slurry can provide

Edited by:

Abdullah Bin Arif
BRIN

Received:

6 February 2025

Accepted:

25 March 2025

Published online:

14 April 2025

Citation:

Anggoro, A. D., Abdillah, M. W., Mullatif, I. A., Widyaningrum, R. P., & Jihan, K. M. (2025). Total glucosinolate content of arugula (*Eruca sativa* Mill.) supplemented with rhizobacteria-enriched bio-slurry. *Jurnal Agronomi Indonesia (Indonesian Journal of Agronomy)*, 53(1), 64-71. DOI:

<https://dx.doi.org/10.24831/jai.v53i1.62496>

nutrients in the form of N-organic which can be absorbed by plants and keep the pH of the planting media stable (Bonten et al., 2014). The nutrient content in cow dung bio-slurry includes complete macro and micronutrients that can be used as organic fertilizer (Turusy et al., 2019). In addition, rhizobacterial substitution spurs maximum nutrient provision through improved growing media conditions that trigger accelerated plant growth (Murtadho et al., 2016). The application of bio-slurry which is appropriately enriched with rhizobacteria has the potential to improve the growth response and biosynthesis of glucosinolate of arugula. This research aimed to determine the total glucosinolate content in arugula due to the application of bio-slurry enriched with rhizobacteria.

MATERIALS AND METHODS

Research site

This research was conducted from June to October 2023 at Brenjonk Organic Farming Community (800 m above sea level), Trawas District, Mojokerto Regency, East Java, Indonesia. Laboratory activities were carried out at the Plant Physiology Laboratory, Plant Biotechnology Laboratory, Faculty of Agriculture, and Organic Chemistry Laboratory, Faculty of Mathematics and Sciences, Brawijaya University, Malang, East Java.

Experimental design

This research was conducted using a randomized complete block design with a single factor consisting of 9 levels, namely *Pseudomonas* + 0 mL bio-slurry (P₀), *Pseudomonas* + 100 mL bio-slurry (P₁), *Pseudomonas* + 200 mL bio-slurry (P₂), *Bacillus* + 0 mL bio-slurry (P₃), *Bacillus* + 100 mL bio-slurry (P₄), *Bacillus* + 200 mL bio-slurry (P₅), *Pseudomonas* + *Bacillus* + 0 mL bio-slurry (P₆), *Pseudomonas* + *Bacillus* + 100 mL bio-slurry (P₇), *Pseudomonas* + *Bacillus* + 200 mL bio-slurry (P₈). Each treatment was repeated 3 times, resulting in 27 treatment plot units.

The rhizobacteria used was obtained from the Department of Plant Pests and Diseases, Faculty of Agriculture, Brawijaya University. Rhizobacteria consisted of *Pseudomonas* sp. 10⁸ CFU mL⁻¹ and *Bacillus* sp. 10⁸ CFU mL⁻¹. Bio-slurry was obtained from 'Biru-Slurry', it was a liquid slurry that came from cow dung sap that had been fermented and squeezed. The nutrient content in the bio-slurry was 15.18% C-organic, C/N ratio 14.05, 6.08% Nitrogen (N), 5.03% P₂O₅, 0.44% K₂O, 5.40 ppm available Fe, and 314.18 ppm Zn.

The original bio-slurry was dissolved with water through several different ratios according to the treatment, i.e., 100 mL bio-slurry: 1,000 mL of water, and 200 mL bio-slurry: 1,000 mL of water. The diluted solution was applied to the planting media through soil dressing immediately after planting of arugula. The solution per polybag was 100 mL, based on the adjustments from the field capacity of the planting media. Rhizobacteria application was given in the form of a solution with a dose of 30 mL per polybag applied at 7 days after planting.

The planting media for arugula was a mixture of soil, manure, and husk charcoal (2:2:1; v/v). The polybag sized 30 cm x 30 cm was filled with a total media of about 10 kg. Polybag was arranged at a distance between plants of 50 cm x 50 cm.

Before transplanting, arugula seeds were sown and maintained for 3 weeks until transplanted into polybags. Decomposer bacteria were added to the media before transplanting. Plant maintenance included watering and weeding was conducted regularly. Pest control uses antagonistic agents. Harvesting of arugula was done at 50-60 days after transplanting (DAP). Harvesting was done by hand picking considering the criteria of the length of the leaves that have bloomed, dark green, and reach a size of ± 10 cm in length.

Data collection

Arugula growth was observed at 7, 14, 21, 28, and 35 DAP on plant height (cm), leaf number, leaf area (cm²), and chlorophyll content (mg mL⁻¹). At harvest, the measurement

included the fresh weight of the whole plant (g), dry weight per plant (g), antioxidant levels (%), and glucosinolate levels (mg g⁻¹).

The antioxidant activity test was tested with the DPPH method following Abbasi et al. (2016), where the measure used the absorbance value of the UV-Vis spectrophotometer at λ 515 nm. The free radical scavenging activity was calculated as a percentage of DPPH color reduction using the equation:

$$\text{Free radical capture activity (\%)} = 1 - \frac{(\text{control absorbance} - \text{sample absorbance})}{\text{control absorbance}} \times 100\%$$

The analysis of the biochemical compound in arugula leaves was carried out by GC-MS method. The glucosinolate evaluation followed Miyazawa et al. (2002) using a spectrophotometric mass detector, while quantification followed the method of Ishida et al. (2012) with some modifications. Glucosinolate compounds have different maximum wavelengths. In the research of Ishida et al. (2012), absorbance was measured at 405 nm, 520 nm, and 425 nm. So, at a wavelength of 425 nm, it shows better accuracy, and the glucosinolates are close to the HPLC results. Glucosinolate quantification used crude extract to which 0.3 mL of PA distilled water and 3 mL of 2 mM PdCl₂ were added. At the last stage, the absorbance value was measured using a UV-Vis Spectrophotometer at λ 425 nm, and quantification of glucosinolate compounds was carried out with the following calibration curve.

The spectrophotometric method estimated the total glucosinolates for each sample. This method was developed by research by Ishida et al. (2012), calculated total glucosinolates (GSL) by substituting the absorbance value at a wavelength of 425 nm (A_{425}) with the following formula:

$$\text{Total GSL}_{(\mu\text{mol/g})} = 305,47 \times A_{425} - 29,66$$

Data analysis

Data were analyzed using analysis of variance (ANOVA) at α of 5% level. The honestly significant difference (HSD) test at the 5% level was performed following a significant treatment effect. In addition, some parameters were also analyzed for correlation levels using simple regression. Data were analyzed using SPSS and Microsoft Excel software for Windows 10.

RESULTS AND DISCUSSION

Arugula growth

Leaf area, chlorophyll content, fresh weight, and dry weight of the whole plant of arugula at 35 DAP were higher with the application of 200 mL of bio-slurry and *Pseudomonas* + *Bacillus*. Those variables were not significantly different from those from 200 mL bio-slurry and *Bacillus* (Figure 1). The growth pattern in leaf area and chlorophyll content in the *Pseudomonas* + *Bacillus* with 200 mL bio-slurry treatment had greater results compared to the rhizobacteria all type treatment enriched bio-slurry all doses (Figure 1). Bio-slurry contained 6.08% N. The high nitrogen content can trigger plant growth and production; Kumar et al. (2023) stated that in each application of 200 mL of bio-slurry with a nitrogen content of 2% into the soil, the available supply is around 4-20 grams of nitrogen per liter of bio-slurry. This indicates that the treatment of 200 mL of bio-slurry enriched with rhizobacteria had the potential to increase available N and symbionts with the roots.

The leaf number and plant length at 35 DAP, were not different among treatments (Figure 1E & 1F). The leaf number of arugula was 51-62 when plants reached the maximum vegetative phase. Arugula growing with the input of bio-slurry have larger leaf numbers than those with no N-organic added (Masitoh et al., 2018). Bio-slurry consists of various organic materials, including manure and other organic matter that have undergone a decomposition process. At the same time, rhizobacteria can help enhance plant growth through various mechanisms such as nitrogen fixation, production of plant hormones, and nutrient solubilization. Bio-slurry is rich in macronutrients and

micronutrients which are essential for plant growth and development. Higher doses of bio-slurry provide a greater supply of these nutrients, promoting better root development, photosynthesis, and biomass accumulation (Hutasoit & Sitanggang, 2018).

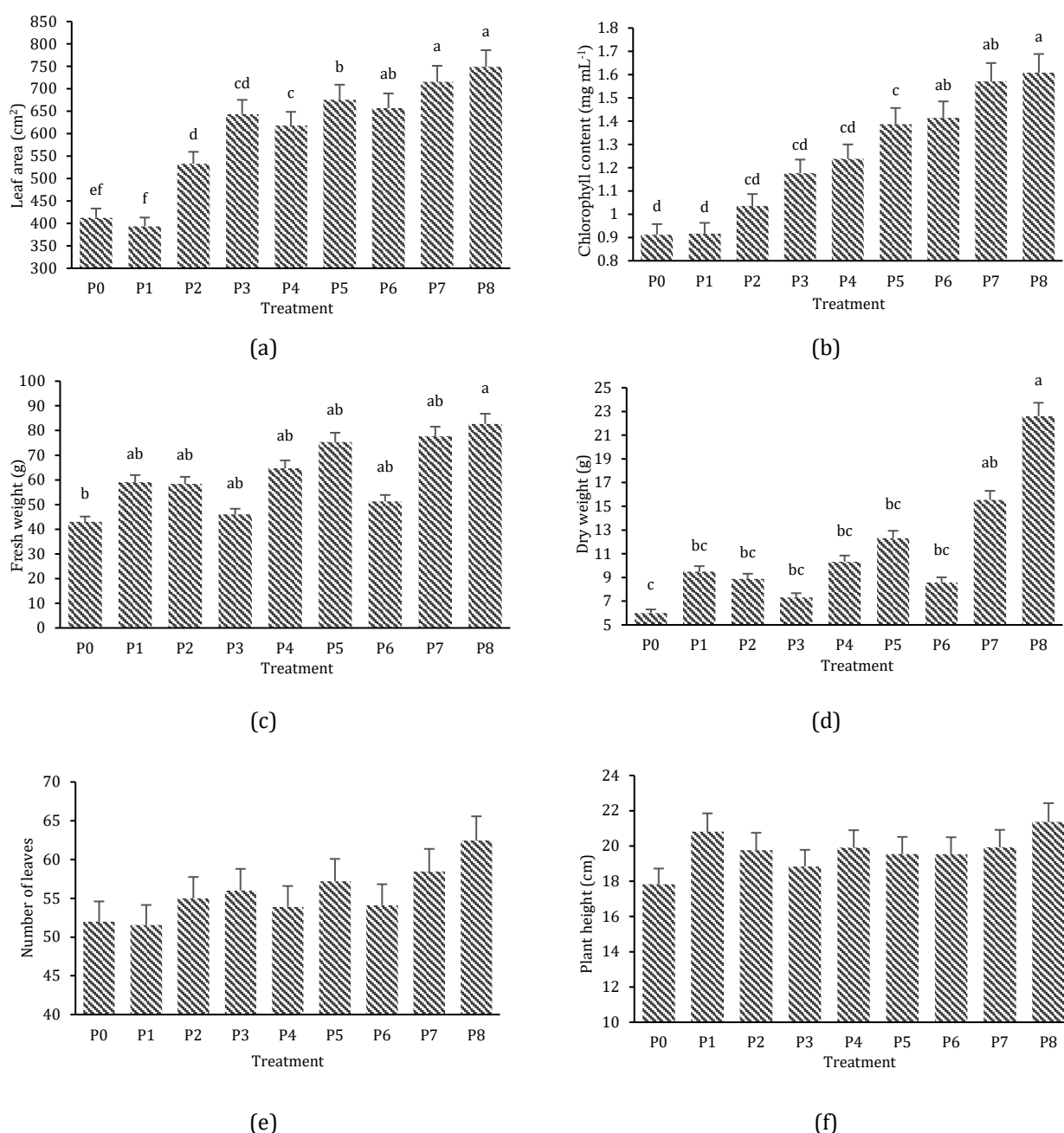


Figure 1. Growth variables of arugula (a) leaf area; (b) chlorophyll content; (c) fresh weight; (d) dry weight; (e) leaf number; (f) plant height. *Pseudoeudomonas* + 0 mL bio-slurry (P₀), *Pseudomonas* + 100 mL bio-slurry (P₁), *Pseudomonas* + 200 mL bio-slurry (P₂), *Bacillus* + 0 mL bio-slurry (P₃), *Bacillus* + 100 mL bio-slurry (P₄), *Bacillus* + 200 mL bio-slurry (P₅), *Pseudomonas* + *Bacillus* + 0 mL bio-slurry (P₆), *Pseudomonas* + *Bacillus* + 100 mL bio-slurry (P₇), *Pseudomonas* + *Bacillus* + 200 mL bio-slurry (P₈).

The synergy between the nutrient-rich bio-slurry and rhizobacteria can optimize the plant's ability to absorb nutrients more efficiently through various mechanisms leading to faster plant growth. Hormones produced by rhizobacteria, such as auxins, can stimulate root and branch growth (Chowdhury et al., 2021). When used together with bio-slurry, their interaction can provide additional stimulation for plant growth and development.

This also influences nitrogen fixation activity in plants, thereby increasing the availability of nitrogen for optimal plant growth (Johnson et al., 2015).

The use of bio-slurry can improve the physical and biological quality of the soil, as well as provide a complete range of macro and micronutrients to support plant growth (Haile & Ayalew, 2018). Meanwhile, rhizobacteria play a role in increasing the availability of minerals and nitrogen in the soil (Hutasoit & Sitanggang, 2018). The use of rhizobacteria, such as *Bacillus* and *Pseudomonas*, can suppress diseases, enhance root nutrient absorption, and promote plant growth (Murtadho et al., 2016). According to de Freitas et al. (2017), arugula is a small plant reaching a height of 15-20 cm at harvest time, with elongated leaves and deeply lobed blades. The leaves are dark green and have a spicy flavor, rich in potassium, sulfur, and iron, as well as vitamins A and C (Khaliq et al., 2021). Bio-slurry contains bioactive compounds and phytohormones produced by beneficial microbes like *Pseudomonas* and *Bacillus*. These compounds stimulate plant growth and enhance stress tolerance.

Phytochemical compound

The results showed that the highest level of free radical scavenging activity was from *Pseudomonas* + *Bacillus* and 200 mL bio-slurry (P8) (Figure 2). Figure 2 shows that the P1 formulation stimulated the lowest antioxidant activity in arugula leaves. According to Abbasi et al. (2016), phenolic compounds and flavonoids were proven to play a significant role in detoxifying free radicals in arugula extract. In the present study, the highest antioxidant activity observed in arugula plants was approximately $\pm 63\%$, suggesting that glucorucin may be a significant contributor to the antioxidant properties of these plants. Further analysis revealed that glucorucin, a prominent glucosinolate compound in arugula, likely plays a key role in enhancing antioxidant capacity, as its breakdown products are known to exhibit strong free radical-scavenging properties.

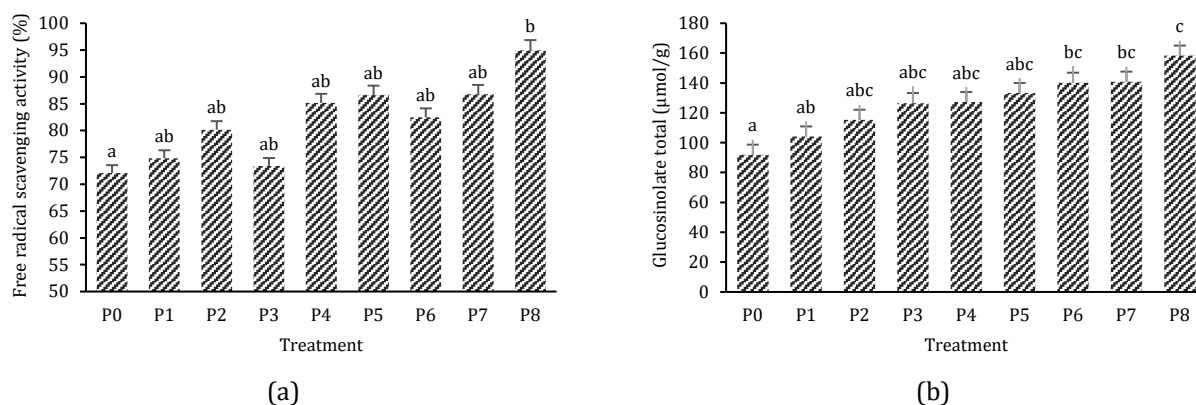


Figure 2. Free radical scavenging activity and glucosinolate in arugula leaves. (a) antioxidant level using DPPH method; (b) total glucosinolate content. *Pseudomonas* + 0 mL bio-slurry (P₀), *Pseudomonas* + 100 mL bio-slurry (P₁), *Pseudomonas* + 200 mL bio-slurry (P₂), *Bacillus* + 0 mL bio-slurry (P₃), *Bacillus* + 100 mL bio-slurry (P₄), *Bacillus* + 200 mL bio-slurry (P₅), *Pseudomonas* + *Bacillus* + 0 mL bio-slurry (P₆), *Pseudomonas* + *Bacillus* + 100 mL bio-slurry (P₇), *Pseudomonas* + *Bacillus* + 200 mL bio-slurry (P₈).

Based on Figure 2, the results show that the highest glucosinolate levels are sequentially in the formulations P₈. Judging from the difference in glucosinolate levels, the difference in levels of these compounds in the 9 treatments was not that big. With this, the combination treatment of rhizobacteria and bio-slurry resulted in a glucosinolate content that was still relatively high (Mitreiter & Gigolashvili, 2021).

Based on GC-MS analysis, it was found 15 chemical compounds (Table 1). The leaf contained a class of furan compounds (Furanpropanoic acid). The isothiocyanate group was not been found in arugula. Compound with the largest area, i.e., 39.22%, and in the

amount of 18.69%, it was identified as compound oxazolidine-2-thione. Based on research by Wijit et al. (2017), the area (%) in the GC-MS data results is expressed as the level of content or concentration of the presence of compounds in the composition of a plant. Hydrolysis of glucosinolate forms oxazolidine- 2-thione, if the hydroxyl group at C-2 of the glucosinolate side chain, then the isothiocyanate is cyclized to produce oxazolidine-2-thione (Wu et al., 2022). However, the main ingredient in the combination of treatments was the compound 2,4,4-Trimethylhexane, with an area of 32.51%, this compound was identified as not resulting from glucosinolate hydrolysis.

Table 1. Phytochemical compound of arugula using GC-MS.

Compound	RT	Area (%)
(R)-2-tert-Butyl-N-((methylthio)methyl)-4-methylidene-5-oxazolidinone	10.16	39.22
Guanidinhydrochlorid	4.19	13.71
1-Chloroethyl acetate	4.48	12.62
Furfuryl alcohol	4.36	10.37
2,6-Dimethylpyrazine	5.21	8.09
2,4,4-Trimethylhexane	24.18	32.51
2,2-Dimethylbutane	23.45	29.26
2,2-Dimethylbutane	23.09	17.19
(Z)-1,3-bis[bis(Diisopropylamino)boryl]-3-phenyl-1- propene	24.34	6.45
Nitromethane	10.16	3.67
R)-2-tert-Butyl-N-((methylthio)methyl)-4-methylidene-5-oxazolidinone	10.17	18.69
Furfuryl alcohol	4.37	15.98
Isocyanic Acid	4.49	12.47
2-Furanpropanoic acid	6.79	8.99
Guanidine carbonate	4.20	8.65

Note: Analysis was performed on a composite sample from all treatments; RT-retention time

Most phytochemical compounds have a potential pharmacological activity that contributes to fighting free radicals or has antioxidant properties (Table 1). All these compounds were subjected to structural comparisons through literature studies to determine the identity and characterization of compounds that have potential properties as antioxidants (Pacifico et al., 2021). In this study, the area percentage value of each compound was observed to estimate the contribution of compound composition to antioxidant properties. Area concentration aims to determine the quantity of the compound contained in a form, whether in plants or other forms, the improved nutrient availability and microbial interactions can enhance the synthesis of secondary metabolites in plants, which are responsible for higher phytochemical content (Wijit et al., 2017).

Based on research conducted by Almushayti et al. (2021), myrosinase can actively react with glucosinolates by cutting, mixing, or chewing, causing hydrolysis of thioglucosides, the formation of glucose and unstable aglycones can occur, and different products can occur spontaneously, such as isothiocyanate compounds (ITC), nitriles, elemental sulfur, thiocyanate, epithionitrile, and oxazolidine-2-thiones (Figure 3). These compounds are responsible for the characteristic pungent taste and odor and the biological activity associated with vegetable consumption (Possenti et al., 2017).

Derivatives of glucosinolate compounds in arugula have potential results in forming antioxidant content. Glucosinolate compounds play a role in developing compounds that have a level of resistance in counteracting free radicals (Elsadek et al., 2021). Glucosinolates and their hydrolysis derivatives (such as isothiocyanates and nitriles) are key bioactive compounds that significantly influence the phytochemical profile, flavor, and potential health benefits of arugula. Their composition varies depending on genetic factors, environmental conditions, and postharvest processing, ultimately affecting the plant's nutritional and sensory qualities. These metabolite compounds are unique, especially in the Brassicaceae, so they can make plants nutritious because their phytochemical compounds provide a good response to human health (Anggraito et al., 2018).

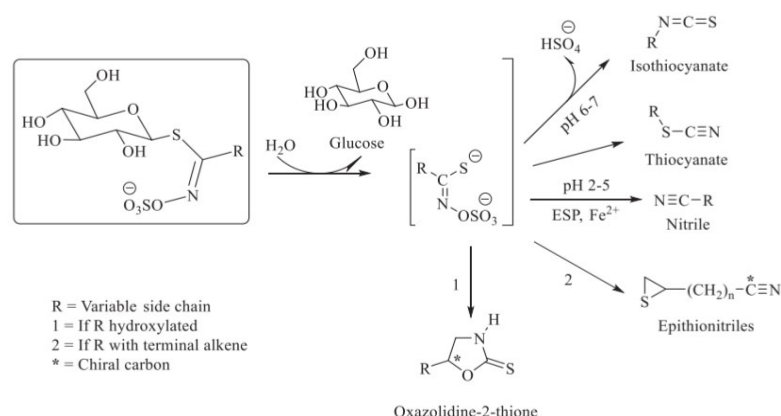


Figure 3. Enzymatic degradation mechanisms of glucosinolates and their hydrolysis products (Almushayti et al., 2021).

CONCLUSIONS

The combined application of bio-slurry and rhizobacteria (*Pseudomonas* and *Bacillus*) increased the glucosinolate content in arugula. Bio-slurry had high organic N reaching 6.08% which might contribute to arugula growth. The highest antioxidant activity value was 96% in the combination of *Pseudomonas* + *Bacillus* + 200 mL bio-slurry. Therefore, it is recommended to apply a 200 mL dose of bio-slurry in combination with *Pseudomonas* + *Bacillus* for optimum arugula growth. Further physiological research is needed to understand the biochemical mechanism of the application of bio-slurry in combination with rhizobacteria on plant growth and development.

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