Junior Science Communications
Faculty of Applied Sciences, UiTM Shah Alam
https://journal.uitm.edu.my/ojs/index.php/JSC

E-ISSN 2637-0689
Vol 43 2025

Colloquium on Applied Sciences 2 2024
8-14 July 2024, Faculty of Applied Sciences, UiTM Shah Alam, Malaysia

Assessment on Optimum *Azolla* Cultivation and Bioactive Properties of *Lactobacillus*-Fermented *Azolla*

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Structured Abstract

Background: The Malaysian poultry industry faces urgent challenges due to the increasing cost of imported feed. In addition, the presence of *Escherichia coli* which is responsible for colibacillosis in poultry can decrease broiler's meat quality. Furthermore, due to its physiological characteristics, susceptibility towards heat stress is becoming a growing concern in the poultry industry since oxidative stress can interfere with the immune system which will lead to death. Thus, exploring new and low-cost food alternatives is crucial. One of the livestock food alternatives researched by many experts is the water fern *Azolla*. This plant was proven to contain various nutritional properties including protein, essential amino acids, minerals, and vitamins. Additionally, fermentation with *Lactobacillus* was also proven to enhance the bioactive properties of its fermentation products. This is due to this bacterium's ability to produce bacteriocin and aid enzymatic reactions that release bioactive compounds from its substrate. Hence, this study intends to investigate the optimum cultivation for *Azolla* in Malaysia's climate, isolate *Lactobacillus fr*om a common probiotic drink, and evaluate the bioactive compounds of the *Lactobacillus*-fermented *Azolla.*

Methods: This study involves both fieldwork and laboratory work. Initially, *Azolla* was cultivated under different parameters and *Lactobacillus* was isolated and identified from a common probiotic drink. Furthermore, the isolated *Lactobacillus* was used to ferment *Azolla* via solid-state fermentation where the bioactive properties of both fermented and unfermented *Azolla* were then evaluated.

Results: The result of this study suggests that *Azolla* can be cultivated in Malaysia's climate. Next, the *Lactobacillus* was isolated and identified from the common probiotic drink. Moreover, the bioactive properties of both fermented and unfermented *Azolla* were determined.

Conclusion: Overall, these findings suggest that *Azolla* can be cultivated under optimal environmental conditions in the Malaysian climate, and isolation of *Lactobacillus* from probiotic drinks is successful. However, fermentation of *Azolla* using *Lactobacillus* does not significantly improve the bioactive properties of *Azolla*. Extensive studies are highly recommended to fully explore the potential of *Lactobacillus*-fermented *Azolla* in improving *Azolla* bioactive profiles to be used as prospective broiler feed.

Keywords: *Azolla* cultivation, *Lactobacillus*, fermentation, bioactive properties

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