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The impact of mangrove plantation in ponds on the secondary metabolite content

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1. Introduction

During the last few decades, planting mangrove in pond and shore areas has been altered in order to obtain various benefits, such as environmental quality control, physical protection, as well as the ecosystem services in order to improve the productivity of traditional fish/shrimp culture [1]–[3]. However, mangrove plants may need to adapt the new environment in order to survive. Moreover, the new environment may have significantly different condition to its requirement. Thus, the planted mangrove plant may achieve stress during the adaptation.

The growth of mangrove species in the coastal area is distributed in zones, including finging zone, intermediate (transition) zone and landward (scrub) zone [4], [5]. The fringing zone is the area that is directly exposed to tides and sea waves. The zone is dominated by pioneer mangrove specieses, such as *Avicennia marina* and Rhizophora mucronata [6]. Both species are the most common mangrove planted in the silvofishery pond due to its tolerance to inundation [7], [8].

Mangrove is vegetation which lives in the coastal area. However, each species require different salinity regimes. *A. marina* is one of pioneer mangrove species which has high tolerance to various environmental condition, such as salinity and soil texture [9]. *A. marina* grows in areas with various substrate, such as sandy to the muddy soil [10]. *A. marina* could even survive in the environment with low dissolved oxygen contentration [11].

Even though some mangrove species could be planted in the ponds, the potential of stress due to infavorable environmental condition may occur. Various aspects such as salinity, temperature, inundation frequency and period, soil texture, as well as nutrient availability and accumulation rates could be the potential threat to the growth of mangrove plants in silvofishery pond [12]. Thus, mangrove plants are forced to adapt to the stressful environment.

Any disturbance on the vegetation leads to the change of metabolism [13]. In order to be able to adapt to the new environment, plants need to adjust its physiological processes through the adjustment of certain chemical compound production. The metabolites are chemical compounds produced by metabolism process. Thus, the adaptation process of mangrove plants may induce evolution in secondary metabolite production [14], [15]. Secondary metabolites are synthesized for various functions, including as a protection against environmental disturbances [16]. Produced metabolites are accumulated in plant's organs with variable rates. The accumulation of secondary metabolites in plants is affected by environmental condition [17].

In certain cases, stress in plants are expected in order to improve the content of certain metabolites [18]. The purpose is to improve certain characteristic of the plants, fruits, or some other specific organs. Improved metabolites production in plants are also expected to obtain mass production of certain chemical compound from plant's extract [19]. This is due to the increased utilization of secondary metabolites in various industries, such as pharmaceutical.

Alkaloids, phenol and terpenoids are three major secondary metabolites produced by plants [20]. Alkaloids is secondary metabolite which is related to the plant development and is much exploited [21], [22]. Alkaloid is used for various medication, such as Alzheimer, Huntington, Parkinson, stroke, Schizophrenia and epilepsy [23]. While phenol is mostly used as anti-oxidant and anti-inflamatory [22], [24]. Terpenoid is the most diverse compound [25]. Potential uses of terpenoid are including food additive, medicinal and biofuels [26], [27].

Considering the current trend of mangrove planting, arise the needs to identify the physiological responses of mangrove plants in silvofishery ponds. The research aimed to analyze the metabolite content in mangrove root and leaf of *Avicennia marina*, to analyze the difference of metabolite content between pond and shore area, and to analyze the correlation of metabolite content between the root and the leaf.

2. Material and methods

The research was carried out in Mangunharjo Village, Tugu District, Semarang City in July 2019. The study was focused on the secondary metabolites content of *Avicennia marina*. Data collection was carried out through one time sampling at the pond and shore areas. Three mangrove tree were occupied at each location. The samples were collected from two mangrove organs, the root and the leaf. Thus, there were total of twelve samples obtained for the research. The weight of the sample was 300 gr respectively. The samples were then analyzed in the laboratory for secondary metabolite contents, including alkaloids, phenol and terpenoid. The analysis for secondary metabolite contents were performed in LPPT UGM.

Data analysis was carried out through t-test and correlation. The analysis was carried out separately for root and leaf content. Analysis with t-test was purposed to compare the concentration of secondary metabolites between growing locations, while correlation analysis was purposed to identify the correlation of metabolite content between the root and the leaf.

3. Result

The laboratory analysis on the secondary metabolite content in *A. marina* root and leaf showed positive content of alkaloids, phenol and terpenoid. However, only alkaloids and phenol could be taken for quantitative analysis. The concentration of secondary metabolite in mangrove root and leaf are shown in *Table 1* and *Table 2* respectively.

Table 1. Metabolite content in mangrove root (%b/b)

Growing location	Metabolites	
	Alkaloids	Phenol

Pond	0.13 ± 0.03[a]	1.95 ± 0.40[a]
Shore	0.08 ± 0.02[b]	2.25 ± 0.40[a]

Note: different letters in the same column indicates significant difference

Table 1 shows that A. marina which was grown in pond area had higher content of alkaloids but lower content of phenol compared to the mangrove grown in the shore area. However, according to the t-test analysis, only the difference of alkaloids concentration was significant, while t he difference in phenol concentration was not significant. This shows that the difference of environmental condition affected the alkaloids production in the root. The test resulted t value of 2.878 (p=0.045) and 0.931 (p=0.415) respectively for alkaloid and phenol.

Table 2. Metabolite content in mangrove leaves (%b/b)

Growing location	Metabolites	
	Alkaloids	Phenol
Pond	0.11 ± 0.01[a]	1.50 ± 0.15[a]
Shore	0.16 ± 0.06[a]	1.06 ± 0.17[b]

Note: different letters in the same column indicates significant difference

Laboratory analysis result for secondary metabolites content in the leaf showed that there were differences in the concentration of alkaloids and phenol. The alkaloids content tended to be higher in the shore area, while the phenol concentration was higher in the pond area. Statistical analysis showed significant difference of phenol concentration. T-test resulted t value of -1.615 (p=0.182) and 3.162 (p=0.034) respectively for alkaloid and phenol.

Based on the analysis result as shown in *Table 1* and *Table 2*, there was a contrary trends of alkaloid and phenol content between the root and leaf. This shows that planting mangrove in the pond has significant effect on the concentration of secondary metabolites, such as alkaloids and phenol. The difference in metabolites could be considered as the response of mangrove plants under environmental stress.

Further analysis was carried out to understand the correlation of metabolite content between the root and leaf. The analysis was carried out separately for each growing location. The result of correlation analysis is presented in *Table 3*.

Table 3. Correlation of metabolite content between root and leaf

Location	Correlation	
	Alkaloids	Phenol
Pond	-0.756 (p=0.454)	-0.998 (p=0.042)
Shore	0.911 (p=0.270)	0.759 (p=0.451)
Overall	-0.305 (p=0.557)	-0.381 (p=0.456)

According to the analysis result as presented in *Table 3*, significant correlation was only obtained in phenol concentration for mangrove plants in pond. However, the correlation direction was negative. Thus, the concentration of phenol in *A. marina*'s root and leaf planted in pond were controvert. If the phenol content in the root is high, the content in the leaf would be low, and vice versa.

As for the other components, even though the correlation were not significant, it should be noted that root and leaf has high correlation level, both for alkaloids and phenol. However, the composite analysis for both location indicates low correlation level. This shows that environment condition has significant effect on the secondary metabolite content. Each location provide different metabolite characteristic. Thus, analysis can not be composited.

4. Discussion
The metabolites content of *A. marina* planted in pond area as shown in Table 1 and Table 2 were deviated from the ones planted in the shore area. The alkaloid in the root of *A. marina* planted in the pond area was higher but lower in the leaf compared to the plants in the shore area. However, inversed condition was shown by phenol content, whereas its concentration in root of *A. marina* planted in the pond area was lower but higher in the leaf compared to the plants in the shore area. The significance of the alkaloid concentration in the root and phenol concentration in the leaf indicated the response of *A. marina* toward environmental stress.

In terrestrial plants, alkaloid content is generally higher in the root [28], [29]. However, some mangrove plants indicate reversed condition whereas the concentration of alkaloid is higher in leaf than in the root [30], [31]. Considering the result of the research, there was a possibility that mangrove plants in the pond area were under environmental disturbance. This was proved byt the higher alkaloid content in the root than the leaf.

We suggested that the difference of alkaloid content in the root was induced by environmental stress. However, the insignificant difference of alkaloid content in the leaf indicated that the impact of environmental stress on the leaf was reduced. Inappropriate environmental condition induced modification of root structure, including the development of small sized root [32]. As the impact, the concentration of alkaloid was improved. The result was appropriate to [33] which proved that alkaloid concentration is higher in the smaller roots. Lower alkaloid content in the leaf of *A. marina* planted in pond area was suggested as the impact of reduced salinity stress. Salinity stress is considered as one of the factors affecting the alkaloid concentration [34].

Phenol content was higher in the root than the leaf. Phenol is an important metabolite to protect plants for various stress. However, currently the relation of phenol concentration toward temperature stress is debatable since there exists contradictive result [35]. Some research showed that temperature increase induce the alteration of phenol concent, while some other showed contrary result. Thus, it can only be suggested that temperature is not the only factor affecting phenol accumulation. Disturbance by microorganisms such as fungi and bacteria is one factor which induce the alteration of phenol accumulation [36], [37].

Referring to the concentration of total phenol in the root and the leaf, it could be suggested that the root underwent more intensive stress than the leaf. The moisture and salinity are known to affect the plant's metabolism, reduce protein content and increase phenol content [38]. Increasing nutrient availability to a certain limit in the environment could improve the accumulation of phenol in the leaf [39]. Considering the possibility, there was a chance that nutrient concentration in pond area was higher than in the shore area. Without the effect of wave activity, the nutrient input in the pond area had higher chance to get suspended. Meanwhile, the wave activity in the shore area fluctuates the nutrient availability.

Terpenoid content in the mangrove root and leaf could not be discussed further since the analysis could only be carried out quantitatively. Terpenoid is secondary metabolite with most variative compound [25], [27]. Thus, it existed in nearly all plant species. However, further investigation is needed to understand the effect of mangrove plantation in pond area to the types and concentration of terpenoid.

The result implicates that plantation of *A. marina* in pond area does have significant impact on metabolite accumulation, both in root and leaf. Environmental stress induce the increase of alkaloid accumulation in the root and phenol accumulation in the leaf. However, the correlation analysis result indicated that the correlation of metabolite accumulation between the root and leaf was weak.

5. Conclusion
According to the result, it can be concluded that *A. marina* planted in pond and shore area both contain alkaloid, phenol and terpenoid, however the concentration was varied. Significant concentration difference between metabolite contents in mangrove planted in pond and shore area of was identified, including alkaloid content in the root and phenol content in the leaf. Significant correlation of metabolite

content in the root and the leaf was obtained only for phenol content in mangrove planted in the pond area.

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