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Original Research Article

Effects of Formulation of Soilless Growth Media from *Azolla Pinnata* sp on Water Absorption, Water Loss, and Water Retention

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Abstract: Most plants require soil as a growth media. Soilless media has been introduced to overcome the lack of soil for plant cultivations. An *Azolla* cube was developed as soilless growth media with nutrient-rich and water-storing capabilities through the usage of *Azolla pinnata sp*. *Azolla* cube is a new research product, providing an alternative to the existing products, such as Midorie Pafcal and Jiffy-7 pellets. This research introduced four samples of *Azolla* cubes at different formulations to evaluate the performance of the cubes on water absorption, water loss, and water retention. This product was developed by using two types of binder method: tapioca starch (Cassava starch) and Bentonite clay. Based on the results, sample C held an estimation of 5.94 g ± 0.01 of 10 g of water within 6 hours at room temperature while that of Sample A, B, and D was 5.45 g ± 0.01, 3.37 g ± 0.01, and 4.70 g ± 0.01, respectively. Sample C also lost an estimation of 2.56 g ± 0.01 of water within 2 days of observation. The results also showed that sample C had the highest percentage of moisture content (15.4%) after undergoing four stages of applied pressure, 0 kPa, 1 kPa, 33 kPa, and 1500 kPa, for the water retention test, in which the value of water movement remained constant. In conclusion, *Azolla* cube was shown to be capable of providing the nutrients needed for plants and absorbing more water compared to the current products.

Keywords: Soilless growth media; water absorption; water loss; water retention; *Azolla pinnata* sp

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1. Introduction

Azolla pinnata sp. is a species of fern also known as mosquitoes' fern. This fern is locally distributed in the native range of Africa, Madagascar, India, Southeast Asia, China, Japan, Malaysia, and the Philippines (Mohamed *et al*., 2018). It floats on a water surface and spreads quickly, covering the open area of the surface water. The fern can double its leaf area within seven days under excellent condition of nutrient and temperature. *Azolla* has been used for centuries in the industries, especially as a fertiliser in the rice production in Southeast Asia. The use of Azolla fertiliser contributes numerous benefits to paddy cultivation that are able to reduce the costs for the cultivation of rice and increase the yield of paddy (Hanafey *et al*., 2021). Furthermore, *Azolla* is also used as a feed product for livestock such as poultry, dairy, fishes, goats, sheep, and pigs. Further studies have been carried out for a more comprehensive range of development of *Azolla* as a biofertiliser. *Azolla* possesses the required qualities for the green manure for crops, such as quick growth, large biomass production, high in nitrogen, and ease of decomposition in soil. Moreover, it is also rich in potassium when applied in soil composition. It is capable of absorbing atmospheric nitrogen directly through its symbiotic relationship with Anabaena which performs the biological nitrogen fixation whereas other plants obtain their nitrogen by absorbing it from a complex compound in the soil through the roots (Soo *et al*, 2019).

Initially, soil consists of nutrients such as nitrogen, phosphorus, calcium, and potassium. These nutrient components are needed for plants to grow. As these nutrients reduce in amount due to uptake during plant growth, without proper measures or actions taken, the plant might suffer from nutrient deficiency and eventually stop growing (Han *et al*., 2019). In this matter, the natural supply of nutrients in the soil must be "re-filled". For this kind of situation, a growth medium plays an essential role as an enrichment to replace the lost nitrogen to the soil either organically, such as the compose of organic matters, or . inorganically, including adding minerals made up of pure and inorganic chemicals.

Future agriculture introduces organic soilless media as an initiative to replace the existing soil as the growing media. Peat, coir, and soft-wood pine bark are the common materials used as growing media. Peat encompasses many different types of plant materials that have been partially decomposed under anaerobic, and waterlogged conditions (Bunt, 1988). While it is inevitable that certain problems might occur such as low re-wetting capacity (Michel, 2010), peats generally tend to possess excellent physical, chemical, and biological properties for plant growth (Bragg, 1990 Robertson, 1993; Schmilewski, 2008; Krucker *et al*., 2010). Coir, also known as coir pith coir meal, coir dust or coco peat, is considered as a waste product in the coconut (*Cocos nucifera*) industry (Arenas *et al*., 2020). It consists of dust and short fibres derived from the mesocarp of the fruit. The general physical, chemical, and biological properties of coir have been widely reviewed (Bragg, 1990; Prasad, 1997; Schmilewski, 2008; Nichols, 2013). Similar to peat, coir provides a favourable balance of air and water to the plant roots. In contrast to peat, however, coir has a high re-wetting capacity (Blok & Weaver, 2005). On the other hand, the soft-wood pine bark has a high air holding capacity and it is typically mixed with other components such as peat to improve water retention (Bilderback & Lorscheider, 1995).

An effective soilless growing medium requires a physical structure that creates an appropriate balance of air and water for a healthy root development. A few types of hydraulic properties can be determined to describe how water is absorbed, held, and released by the growing media (Fonteno *et al*., 1990). Water absorption is a process of the capillary forces exerted by the pore structure, causing fluid to be drawn into the body of the material (Neville, 1996). In this study, water absorption test was carried out to determine how the *Azolla* cube could absorb and hold water. After the test of water absorption, other characteristics were tested, and it seemed that there were water losses from the soilless growth media. The loss of water was natural through the evaporation process by sunlight. Besides the evaporation process, the water in the media can be lost during the infiltration process (Lejcuś *et al*., 2015). The capabilities of the growth media to absorb and hold water in a long period of time, and have a slow rate in releasing water help the plants grow well. Particular attention has been paid to the ability of a growing medium to store water or its "water retention characteristic" (Fonteno, 1993). The water retention is the determination of undisturbed soil cores by proceeding through a series of wetting and drainage event in the field by measuring the water content and pressure potentials simultaneously during a transient flow experiment (Nimmo, 1991). In a laboratory study, there are multiple physical relationships and the extraction of soil solutions for the chemical analysis. The Pressure Plate Extractor (SKU1250, Soil Moisture, USA) has become an eminently successful research tool. There was a previous study related to the determination of water retention characteristics of perlite and peat. In the previous study, there were seven samples prepared with different formulations: 100% perlite, 100% peat, 80% perlite + 20% peat, 70% perlite + 30% peat, 50% perlite + 50% peat, 30% perlite + 70% peat, and 20% perlite + 80% peat. All samples would be subjected under seven different pressures: 0.1 bar, 0.33 bars, 1.0 bar, 3.0 bars, 6.25 bars, 11 bars, and 15 bars. Based on the study, higher water retention seen at points of tension of 0.1 bar, 0.33 bars, 1 bar and 3 bars showed percentage of moisture of 67.88%, 58.33%, 47.70% and 39.78% the volume of percentage, respectively. Meanwhile, at 6.5 bars, 11 bars and 15 bars, the percentage of moisture dropped. That study showed perlite had higher moisture content compared to peat.

Most artificial and soilless growth media are formed using chemical products to maintain the health of the soil. Since *Azolla* is a recently developed organic material used in soilless growth media, only a few researches were found. Hence, the purpose of this study is to develop a formulation for the *Azolla* cubes as soilless growth media with water-storing capacity using *Azolla pinnata* sp.

2. Materials and Methods

2.1 Azolla Cube Preparation

Fresh *Azolla pinnata sp.* had been cultivated using vertical multilayer rectangular shelves (VERSMAP) at Laboratory of Machinery Design and Food Processing, Faculty of Engineering, UPM. The matured *Azolla* was harvested and dried under the sun for 4 days. There were four samples of *Azolla* cubes: Sample A, B, C, and D, with one replication for each sample. The *Azolla* cubes were formed by mixing the dried *Azolla* with two types of binders: cassava starch and Bentonite clay. Firstly, dried *Azolla* was divided into two categories comprising of ground and unground *Azolla*. The ground *Azolla* was used in sample A and D. Meanwhile, the unground *Azolla* was used in Sample B and C. The ground *Azolla* was produced using a domestic blender (EBM-B1235 (IV) Blender, ELBA, Italy). After that, the ground *Azolla* was sieved using a 250 µm sieve. Then, all materials were mixed at different ratio in Table 1. All the materials were mixed well with a spoon. The mixture was then poured into a 1 inch cube mould. All samples were placed at room temperature (27°C–30°C) for 3 days to ensure that the mixtures were entirely formed into cubes.

Table 1. Formulation ratio used for the development of the *Azolla* cubes

Samples	Formulation ratio (g)		
	Dried *Azolla* (Grind/ Not grind)	**Binders (Cassava starch/ Bentonite clay)**	**Water**
A	5.5 (ground *Azolla*)	2.0 (Cassava starch)	5.0
B	2.0 (unground *Azolla*)	2.0 (Bentonite clay)	5.0
C	2.0 (unground *Azolla*)	2.0 (Cassava starch)	5.0
D	5.5 (ground *Azolla*)	2.0 (Bentonite clay)	5.0

2.2 Water Absorption Test

The samples were removed from the mould and placed on the crucible dishes. The experiment was conducted to determine the water absorption of *Azolla* cubes. Water absorption is the ability of all samples to absorb an amount of water throughout a period of 6 hours. A total of 10 g of water was dropped onto all samples contained in a crucible dish at room temperature (27°C–30°C) and subsequently observed at every 1 hour intervals for the water absorption process. The samples were taken out periodically and wiped to remove the water from the surface of the sample, and weighed immediately using a balance (710D, WAYMASTER, England) to determine the amount of the absorbed water. The samples were weighed regularly, once every hour for the first 6 hours. Prior to the water adsorption test, the initial weight for each sample was measured. Sample A was 6.75 g, B was 3.07 g, C was 3.22 g, and D was 5.36 g. Then, the weight of the water absorption for all samples were measured and recorded hourly for the next 6 hours. The water absorption weight after 6 hours was measured following Equation (1):

$$\text{Mass of water absorption (g)} = M_2 - M_1 \quad (1)$$

Where M_2 and M_1 are the mass of wet sample and dry sample respectively.

2.3 Water Loss Test

Water loss is a process where the soil loses its water content caused by the evaporation process or when the plants absorb the water through their roots. The total water loss test was carried out through a 2 days observation (48 hours) to obtain the results of the water losses at room temperature of 27°C–30°C. The final mass of water absorption from each sample at section 2.2 will be recorded as initial reading in water loss process. Each sample was placed under observation for 48 hours to take the water loss of all samples. This experiment was a continuous process from the previous experiment (water absorption experiment). The initial mass for all samples were 8.74 g for sample A, 5.94 g for B, 6.35 g for C, and 8.96 g for D. All results are recorded in Table 2. The mass loss of the samples was measured at different time intervals using the following Equation (2):

$$\text{Mass of water loss (g)} = M_1 - M_2 \quad (2)$$

Where M_1 and M_2 are the mass of wet sample and dry sample respectively.

2.4 Water Retention Pressure-Plate Test

Water retention provides a convenient and reliable means of removing soil moisture from soil samples under controlled conditions throughout the entire plant growth period without disturbing the soil structure. In this test, four stages of pressure were applied to all *Azolla* cube sample. This experiment was a continuous process from the water absorption experiment. In order to determine the water retention, pressure plate test was conducted. The aim of this test was to obtain the values of equilibrium moisture content at higher relative humidity levels about 95–100% using specific laboratory technique. At the first stage, 0 kPa force was applied to all samples. This stage is known as the saturated point. In this stage, the samples would be saturated with water content to the extent that the water can no longer be absorbed by the samples. During the second stage or known as a wet point, 1 kPa force was applied. This process was continued until there was no water movement and the weight was constant. Later, 33 kPa of force was applied to all samples during the stage known as the field capacity point. This stage shows the upper limit of water storage. During the final stage, known as the permanent wilting point, 1500 kPa of force was applied to the samples. All results of water retention are recorded in Table 3.

2.5 Statistical Analysis

An Analysis of Variance (ANOVA) test was used to analyse the differences among group means in a sample. The confidence limit was considered as 95% ($p < 0.05$). The values were stated as the mean ± standard deviation.

3. Results and Discussions

3.1 Determination of Water Absorption

In order to find the water absorption of *Azolla* cube samples, the results of the experiment were gathered and interpreted intoin Table 2. Table 2 showed the average mass of Sample A, B, C and D within 6 hours, in which the readings were taken a total of three times. From Table 2, at the first hour, the water absorption mass of sample A increased in amount, from 6.75 g ± 0.05 to 13.47 g ± 0.05. During the second to fourth hour, the weight of water absorption slightly increased from 13.53 g ± 0.05 to 13.65 g ± 0.05, and thereafter, dropped to 13.60 g ± 0.05. Then, during the fifth and sixth hour of observation, the mass of water absorption dropped further from 12.82 g to 12.20 g. Based on the mass of water absorption for sample A, it showed that the water could be absorbed effectively.

Table 2 indicated that sample B managed to absorb an estimation of 1.5 g of water at hour 1 since the initial mass which were 4.57 g ± 0.05 from 3.07 g ± 0.05 of mass of water from the initial mass. For the following hours, at hour 2 to 6, the mass of water increased steadily, which were 5.13 g ± 0.05, 5.64 g ± 0.05, 6.05 g ± 0.05, 6.15 g ± 0.05, and 6.45 g ± 0.05, respectively. It showed that sample B could still absorb and held water steadily until it reached the hour 6 of the observation which known as maximum limit of water capacity that could be absorbed.

Based on the data provided in Table 2, the mass of water absorption for sample C throughout the experiment showed an increment of 3.22 g ± 0.05 to 10.41 g ± 0.05 during the hour 1 of observation. During the hour 2 and 3 of observation, the mass value gradually decreased from 10.43 g ± 0.05 to 10.31 g ± 0.05. As for the final 3 hours, the reading of weight dropped steadily from 9.78 g ± 0.05, 9.43 g ± 0.05, and 9.16 g ± 0.05, respectively. Sample C ability to absorb water quickly might be due to more air pores rather than to hold the water in an extended period.

The result for sample D was shown in Table 2. The mass of sample D increased from 5.36 g ± 0.05 to 9.96 g ± 0.05 during the first 1 hour of observation. Then, the mass increased slightly from 9.96 g ± 0.05 to 9.98 g ± 0.05. The weight remained stable at 9.98 g ± 0.05 during the hour 2 to 4. The mass showed another slight increment at hour 5, when it increased from 9.98 g to 10.00 g, and eventually stopped at 10.06 g during hour 6 of the observation. Sample D had the slowest absorption rate within 6 hours.

Table 2 shows the standard deviation value of water absorption for *Azolla* cubes within 6 hours. Sample A, which was made up from ground *Azolla* and cassava starch had the initial weight of 6.75 g. It then achieved a final weight of 12.20 g after the hour 6 of observation. The difference of weight was significant with a value of 2.30 of water absorption. Sample B was made up by unground *Azolla* and Bentonite clay. It had an initial weight of 3.07 g and subsequently reached a final weight of 6.44 g. The result showed that Sample B managed to absorb 3.37 g of water. The difference of weight was significant with a value 1.09 of water absorption. Sample C was made from unground *Azolla* and cassava starch. It could absorb a total of 5.94 g water, from an initial weight of 3.22 g to a final weight of 9.16 g. The difference of weight was significant with a value 2.39 of water absorption. In the end, Sample D absorbed 4.70 g of water, from 5.36 g to 10.06 g. The difference of weight was significant with a value 1.62 of water absorption. Based on the average weight of water absorption of samples, Sample C had the highest weight of water absorption of 5.94 g compared to Sample A, Sample B, and Sample D, which were 5.45 g, 3.37 g, and 4.70 g, respectively. According to the results, all samples had significant values with Sample Chaving the highest difference value, showing that Sample C had the best water absorption.

Table 2. The average mass of samples after absorbing from given 10 g of water.

Samples	**Time (hour)**						
	Initial	***1st***	***2nd***	***3rd***	***4th***	***5th***	***6th***
	Mass of samples (gram)						
A	6.75 ± 0.05	13.47± 0.05	13.53 ± 0.05	13.65 ± 0.05	13.60 ± 0.05	12.82 ± 0.05	12.20 ± 0.05
B	3.07 ± 0.01	4.57 ± 0.05	5.13 ± 0.05	5.64 ± 0.05	6.05 ± 0.05	6.15 ± 0.05	6.44 ± 0.05
C	3.22 ± 0.05	10.41 ± 0.05	10.43 ± 0.05	10.31 ± 0.05	9.78 ± 0.05	9.43 ± 0.05	9.16 ± 0.05
D	5.36 ± 0.05	9.96 ± 0.05	9.98 ± 0.05	9.98 ± 0.05	9.98 ± 0.05	10.00 ± 0.05	10.06 ± 0.05

Data are expressed as mean ± SD. Different letters indicate statically significant differences exist $p > 0.01$ for each column. Means that do not share a letter represent significantly different One-way ANOVA test was applied with 95% simultaneous confidence intervals.

3.2 Determination of Water Loss

In order to determine the water loss of *Azolla* cube samples, the results of the experiment were gathered and recorded in Table 3. Table 3 showed the results of an average mass of water loss within 2 days at room temperature of 27°C–30°C and the standard deviation obtained for sample A, sample B, sample C, and sample D. As shown in Table 3, the initial mass of sample A was 8.74 g ± 0.01. After 2 days of observation, the mass of sample A became 5.86 g ± 0.01. The water loss in Sample A was approximately 2.88 g ± 0.01 in 2 days. The difference of mass was significant with a p value less than 0.05 of water loss. Meanwhile, the results of water loss for sample B were about 2.49 g ± 0.01, indicating that the final mass dropped from 5.94 g ± 0.01 to 3.45 g ± 0.01 after 2 days of observation.

The difference of mass was significant with a value $p < 0.01$ of water loss. As for sample C, the water mass loss dropped from 6.35 g ± 0.01 to 3.79 g ± 0.01 after 2 days of observation. The difference of mass was significant with a value $p < 0.01$ of water loss. Similarly, the result of water loss of sample D showed that the difference of weight was significant with a value $p < 0.01$ of water loss. The initial mass was 8.96 g ± 0.01 and then dropped to 5.63 g ± 0.01 after 2 days of observation. Based on the results tabulated in Table 3, all samples demonstrated significant values of water loss to a certain extent within 2 days of observation.

Throughout this experiment, sample A, which was made up from dried ground *Azolla* and cassava starch, had an initial mass of 8.74 g ± 0.01. Then, it achieved a final mass of 5.56 g ± 0.01 after 2 days (48 hours) of observation, showing that sample A had lost 5.45 g ± 0.01 of water. Sample B which was made up of unground dried *Azolla* and Bentonite clay, had an initial mass of 5.94 g ± 0.01 and it reached a final mass of 3.45 g ± 0.01, indicating a loss of 2.49 g ± 0.01 of water. Sample C which was made from unground dried *Azolla* and cassava starch, showed a loss of 2.56 g ± 0.01 after having an initial weight of 6.35 g ± 0.01, resulting in a final mass of 3.79 g ± 0.01. Lastly, sample D had lost 3.33 g ± 0.01 of water, decreasing from 8.96 g ± 0.01 to 5.63 g ± 0.01. Based on the average weight of water loss, sample C had the lowest weight of water loss (2.56 g ± 0.01) compared to sample A and sample D, which were 2.88 g ± 0.01 and 3.33 g ± 0.01, respectively. Besides that, according to the results, it could be concluded that sample A and sample C, which used cassava starch as a binder, had a consistent performance to loss water compared to sample B and sample D which used Bentonite clay as a binder. All samples obtained significant values of water loss with $p < 0.01$. Sample A and sample D lost more water compared to sample B and sample C, but when comparing sample B and sample C, the difference between the both of them was at a slight value of 0.07 g.

Table 3. Average mass of water loss (g) of *Azolla* cubes within 2 days (48 hours).

Samples	**Time (hour)**		**Water loss (g)**
	24	48	
	Mass of sample (gram)		
A	8.74 ± 0.01^{b}	5.86 ± 0.01^{a}	2.88 ± 0.01^{b}
B	5.94 ± 0.01^{d}	3.45 ± 0.01^{d}	2.49 ± 0.01^{d}
C	6.35 ± 0.01^{c}	3.79 ± 0.01^{c}	2.56 ± 0.01^{c}
D	8.96 ± 0.01^{a}	5.63 ± 0.01^{b}	3.33 ± 0.01^{a}

Data are expressed as mean ± SD. Different letters indicate statistically significant differences $p < 0.01$ for each column. Means that do not share a letter represent significantly different. One-way ANOVA test was applied with 95% simultaneous confidence intervals.

3.3 Determination of Water Retention

Table 4 shows the results of water retention for Sample A, Sample B, Sample C and Sample D in wet basis condition. Based on the result, Sample C had the highest value of moisture content (18.34% ± 0.01) compared to Sample A, Sample B and Sample D which were 5.83% ± 0.05, 7.57% ± 0.05, and 10.84% ± 0.01, respectively. When the 0 kPa pressure plate was applied to all samples, the percentage of moisture content was still relatively high, indicating that all samples had reached their maximum saturated point. In this condition, no additional water can be absorbed by the samples as it has reached the maximum point although it could still hold the water. Based on Table 4, when the 1 kPa pressure plate was applied to all samples, the percentages of moisture content for Sample A, Sample B, Sample C and Sample D were 12.05% ± 0.01, 11.21% ± 0.01, 24.19% ± 0.01, and 18.23% ± 0.05, respectively. In this particular condition, all samples remained in a wet condition as the cubes were still comprised of water. This process was continued to the point that there was no water movement and the weight was constant. During the third stage, 33 kPa of pressure plates were applied to the samples, showing that the percentage of moisture content in Sample A was 5.83% ± 0.05, Sample B was 7.57% ± 0.05, Sample C was 18.34% ± 0.01, and Sample D was 10.84% ± 0.01. In this study, the third stage was critical as it focused on the field capacity points of the samples. At this point, the results showed the upper limit of water storage before the sample showed the lower limit of water storage also known as the permanent wilting point. At the field capacity point, samples could hold and store water. During the last stage, all samples were applied with 1500 kPa to determine the value of moisture content when the samples were at a permanent wilting point stage. From the result, Sample C still had the highest percentage of moisture content (15.40%) in comparison to Sample A, Sample B and Sample D which were 26.11% ± 0.05, 62.53% ± 0.05, and 29.37% ± 0.05, respectively. Based on the water retention test, it could be concluded that Sample C was the most appropriate sample of *Azolla* cube to be used for water storing.

Table 4. The final result of water retention for all samples of *Azolla* cube.

Samples	**Moisture Content percentage (%) (w/w wet basis)**			
	0 kPa	1 kPa	33 kPa	1500 kPa
A	16.40 ± 0.01^{d}	12.05 ± 0.01^{c}	5.83 ± 0.05^{d}	26.11 ± 0.05^{c}
B	28.45 ± 0.01^{a}	11.21 ± 0.01^{d}	7.57 ± 0.05^{c}	62.53 ± 0.05^{a}
C	27.04 ± 0.05^{b}	24.19 ± 0.01^{a}	18.34 ± 0.01^{a}	15.40 ± 0.05^{d}
D	24.90 ± 0.01^{c}	18.23 ± 0.05^{b}	10.84 ± 0.01^{b}	29.37 ± 0.05^{b}

Data are expressed as mean ± SD. Different letters indicate statistically significant differences $p > 0.01$ for each column. Means that do not share a letter represent significantly different. One-way ANOVA test was applied with 95% simultaneous confidence intervals.

4. Conclusion

All samples have been tested with several analyses to ensure that the appropriate amount of data and information was collected for each of the samples. The analyses performed on the samples were water absorption (g), water loss (g), and water retention (%). Based on the results from water absorbing test, Sample C was deemed to be the best, with the highest rate of water absorption (5.94 g). As for the water loss test, Sample C had only a loss of 2.56 g, which was slightly closer to sample B, which had lost 2.49 g. As such, Sample C was considered to be the better sample, solely based on the results of the water absorption. As for water retention test, Sample C had the highest moisture content percentage (%) (w/w) of 15.40% ± 0.05 at a pressure of 1500 kPa. Based on all results, it can be concluded that Sample C is the most suitable formulation as a soilless medium due to its water-storing capacity, with a significant value in water absorption, low water loss and high percentage in moisture content. Sample C could absorb water quickly with a slow rate of water loss and were able to store the highest value of moisture content. More research and development are required for further improvement to increase the efficiency of *Azolla* cubes in all aspects and to ensure that they can be recognised within the ever-growing soilless media field for the agriculture industry.

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