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THE EFFECT OF EGGSHELLS AS A GROWING MEDIUM TREATMENT ON PLANT GROWTH PERFORMANCE OF RED SPINACH (*Amaranthus dubius*)

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Abstract:

Poultry waste, like eggshells, has been piling up on Earth through the years. On the other hand, home gardening has been a world trend as everyone urge to stay at home during this pandemic. This study aims to observe the influence of different percent of eggshells in a growing medium on the growth performance of red spinach (*Amaranthus dubius*). The eggshell fertilizer used in this study was collected, dried, crushed, and sieved before being used in the experiment. Three treatments were conducted: 100% cocopeat (blank), 50% cocopeat with 50% eggshells, and 80% cocopeat with 20% eggshells. The experiment was carried out over 35 days, with measurements of the plant's height and number of leaves taken every five days. The chemical analyses of ICP-OES and FTIR on eggshells and cocopeat were performed to determine the nutrient content. The result from ICP-OES shows that Calcium (Ca) showed the highest minerals in the eggshell. Blank medium shows the highest growth yield with mean height, leaf number, and fresh weight of 29.49 cm, 17.38 and 16.5g, respectively. While the result of a high percentage of eggshells shows 50% of eggshells forbid the growth of plants. The data were analyzed using One-Way ANOVA to determine the percentage regression and p-value. The results of this study could lead to further investigation of the suitable amount of eggshell in the soilless medium growth.

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Keywords:

Soilless Agriculture, ZESA System, Amaranthus Dubius, Sustainable Agriculture, Horticultural Plants

Introduction

Red spinach (*Amaranthus dubius*) is a leafy vegetable native to South America that has spread to South and Southeast Asia and Africa (Yong et al., 2017). This plant is productive and adapts to various edaphoclimatic conditions, particularly dry and hot temperatures (Molina et al., 2015). *Amaranthus dubius* has a high yield in green matter, excellent nutritional content, and a low proportion of anti-nutrients (Molina et al., 2018). A recent study discovered that red spinach can be used as an alternate source of fibre and protein in rabbit feed formulations (Molina et al., 2018). Furthermore, *Amaranthus dubius* is a staple in many rural communities, where the leaves and tips of red spinach are frequently eaten as salads or stir-fried (Yong et al., 2017). The leaves and stems also have therapeutic characteristics that can help with constipation, anaemia, kidney disorders, and stomach-aches (Sigamoney et al., 2016).

Sustainable Development Goals 2 related to ending hunger, achieving food security, improved nutrition and promoting sustainable agriculture. In 2020, about 149.2 million children under the age 5 suffer from stunting. Lack of nutrition is the one factor that contributes to children stunting all over the world (Wang et al., 2022). Therefore, to achieve the target in order to end all forms of malnutrition and develop sustainable agriculture, growing our own food using modern farming is the best solution.

Like other plants, red spinach needs some nutrients to grow perfectly. Plant nutrition and nutrient ratios are essential factors impacting growth physiology and agricultural crop production yield (Mardanluo et al., 2018). A total of 16 essential nutrients are needed by the plant, which it receives from the atmosphere and soil water. The nutrients are carbon, hydrogen, and oxygen. At the same time, the remaining elements required are nitrogen, potassium, phosphorous, calcium, sulphide, etc. (Khairnar & Nair, 2019). All these nutrients are provided either by adding fertilizer to the soil or by nutrient-rich soil.

The soilless culture is internationally known for its ability to facilitate the productive and intensive development of plants. Using soilless mediums such as cocopeat will overcome various soil media limitations. Cocopeat, with such beneficial properties, is not recommended for agricultural use due to its high lignin and cellulose content (College et al., 2015). Even with the same appearance and texture as soil, cocopeat should be used with other agricultural manure or fertilizer to create an excellent crop medium. Though, there have been many issues with the use of excess fertilizer. Excess fertilizers alter the soil since it contains too much salt, which may destroy beneficial soil microorganisms (Rahman & Zhang, 2018).

Few initiatives have been done to reduce the excessive use of fertilizer, one of which is substituting it with eggshells. An Eggshell is a chain of calcium, magnesium carbonate and calcium phosphate crystals-associated protein fibre (Amu et al., 2008). Waheed et al. (2019) state that it represents nearly 98.2 % calcium carbonate, and the remaining is magnesium and phosphate (Adogla et al., 2016). Discarded eggshells are often used as a plant fertilizer as the calcium in eggshells was found to be similar to the common soil additive (lime), that it could

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be the best natural source of calcium for plants (Wazir et al., 2018; Radha & Karthikeyan, 2019).

Eggshell fertilizer is cheap and environmentally friendly, as material for plant growth is reused by the process. Commey & Mensah, (2019) said that calcium from eggshells can elevate the pH level of highly acidic soil much like calcareous, thus making it favourable for planting (Waheed et al., 2020). Besides, eggshells are the lowest toxic substances available compared to other natural calcium sources. The use of eggshells also helps in soil stabilization. It aims to improve soil engineering properties, which may include increased soil density, cohesion, friction resistance and reduced plasticity index. Thus, chicken eggshells would be used as a substitute since they have the same chemical composition, making it a good soil amendment material (Adogla et al., 2016).

Red spinach requires a lot of nutrients and minerals from the soil to develop well. Typically, using soil as a medium necessitates the addition of fertilizer or manure to ensure an adequate nutrient supply for plant growth. Nutrients and water are probably the agricultural elements that limit plant growth and yields. However, fertilizer application into the soil must be made appropriately, or it may have a detrimental effect. Excessive fertilizer use raises production costs and contributes to the emergence of environmental damage. Excessive rains can leach fertilizer from the soil, returning it to its nutrient-depleted state. Farmers tend to use more fertilizer, which raises production costs and is a major source of river pollution. The utilization of soil as a growing medium necessitates an ongoing watering operation. This increases farmers' costs and the nuisances process for house plantation. Because plants need to collect all their nutrients from soil, various approaches have been created to improve or adapt soil to fit the plant's demands.

Therefore, the soilless medium-growth technology like hydgrowpipe is highlighted in this work. This approach works largely to prevent excessive fertilizer and water for planting by introducing cocopeat as a growing media. Additionally, it overcomes the limitation of the planting site and is perfect for a home plantation. In addition, several studies have been carried out relating the growth of plants using natural sources of mineral or green fertilizer on red spinach. Eggshells contain calcium, a nutrient the plant requires to grow (Waheed et al., 2020). The use of eggshells thus makes them an inexpensive and environment-friendly fertilizer. However, monitoring of eggshells' use on Amaranthus dubius growth inside a hydgrowpipe has not yet been reported. Hence, the aim of this study focuses on the effect of eggshells as a growing medium treatment on the performance growth of *Amaranthus dubius* using the hydgrowpipe system.

Literature Review

Red Spinach (Amaranthus Dubius)

Amaranth is a plant belonging to the *Amarantaceae* family, genus *Amaranthus*, and has over 60 species found in tropical and subtropical areas (Mujaffar & Lee Loy, 2017; Molina et al., 2018; Molina et al., 2015). *Amaranthus dubius* is a tropical and subtropical American plant native to Central and South America. It is widely distributed in Venezuela and other tropical and subtropical American countries (Molina et al., 2018). This plant is regarded as a weed of different crops, like maize, sorghum, and various legumes (Molina et al., 2015). Like leafy greens, red spinach post-harvest life is relatively limited due to quick wilting in tropical

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environments (Mujaffar & Lee Loy, 2017). Molina et al. (2015) state that *Amaranthus dubius* has a large production of green material about 4200 kg dry matter [DM], 263 and 205 g/kg DM of crude protein (CP) respectively in leaves and panicles, and 333 and 230 g/kg DM of crude fibers (CF) in stalks and panicles. *Amaranthus dubius* leaves and stems are part of the food of many rural people who also benefit from the plant's medicinal benefits in treating constipation, anemia, kidney issues and stomachache (Sigamoney et al., 2016). In every country, the use of *Amaranthus dubius* is often varied. In Tanzania, the whole plant is utilized as a stomach discomfort treatment. *Amaranthus dubius* is used in Uganda in potash production. Taiwanese aborigines which are from the Hualien region use this plant's leaves and stems as a type of pot herb (Jung et al., 2008). Sigamoney et al. (2016) state that stalks and leaves contain oxalates, nitrates, and many phenolic chemicals (gallic acid, rutin, ferulic acid, caffeic acid or quercetin) which may help reduce Ag^+ in $AgNO_3$ in nanoparticles produced by Ag. Due to the excellent nutritional composition of some amaranth species, their existence has made them important in humans and animals (Molina et al., 2018). Iv et al. (2021) state that researchers recently found that a new dietary NO_3 supplement may be consumed with the red spinach extract (RSE). RSE is a rich food source of NO_3 that increases plasma levels of NO_3/NO_2 30 minutes after consumption. In addition, a 1 g RSE dose significantly increased the air threshold during the graded exercise starting at 65–75 minutes after ingestion compared to placebo with maltodextrin (PL). A study also found that *Amaranthus dubius* in the feed formulation of rabbits could become a potential substitute for conventional raw materials. It can replace conventional ingredients in rabbit diets to levels up to 32% without adversely affecting high-quality carcass or meat quality (Molina et al., 2018).

Plant Growth

In general, growth should mean an irreversible increase in the weight, volume, area or length of the plant or tissue or organ of the plant. A growth factor may be defined as a collection of substances, any of which, when added in sufficient quantities, will promote the continued growth of a particular system (Pandey et al., 2017). Plant development parameters can be measured in terms of their total number of leaves, total number of branches, height of plants, and soil properties, such as acidity, and pH. These growth parameters such as number of branches and plant height are mainly influence by their water uptake. Inhibited plant growth can be caused by a decrease in soil water absorption and cell turgidity (Al-elwany, 2018). As the irrigation supply is higher, the plants appear to be higher than those with low irrigation supplies (Ratmadanti, 2017). Besides, soil salinity also had a detrimental effect on plant heights and dry weight. Hoang et al. (2020) state that salinity stress inhibited the growth of *Amaranthus dubius*, with the result of a study showing that the root dry weight in red amaranth was greatly reduced, with up to a 75% reduction. Research has also shown that the high salinity of red amaranth has similar reactions, with a 42% decrease in the number of leaves and a 40% reduction in total leaf area (Hoang et al., 2020). *Amaranth* tricolor was more tolerant than the *Amaranthus dubius* compared to the same *Amaranth* family. *Amaranthus* tricolor also produced more phytochemical content under salinity stress than *Amaranthus dubius*, associated to its free radical scavenging properties (Sarker & Oba, 2019). This soil salinity can be solved by soilless media such as cocopeat. Cocopeat is a good growing media variable with adequate pH, electrical conductivity (EC) and other chemical characteristics. Previous research shows that higher leaf counts and fresh and dry leaf weights have been observed in the coconut media (Awang, 2016). Flowering stem height was also significantly affected, and the highest flowering stem height was observed in coconut peat media. Therefore, the beneficial effects of this medium by directly incorporating fertilizer into flowering shrubs have been examined.

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Plant Nutrient

As the population grew rapidly, the meets toward highly efficient food production from agriculture sector desperately needed. Agricultural productivity and food quality depend on the plant nutrition (kulkarni & Goswami, 2019). Each plant has a different set of requirements for nutrients. Below this requirement stage, plants display signs of nutrient deficiency (Moreira et al., 2015). Plants consider two groups of nutrients essential which are micronutrients and macronutrients. Macronutrients are building blocks of essential cellular components like nucleic acids and proteins that require significant quantities. The primary macronutrients are phosphorus, nitrogen, magnesium, and potassium. Carbon, hydrogen, and oxygen are also known as macronutrients as they are necessary to produce the greater organic molecules in cells, but they are non-mineral macronutrient class. Micronutrients, including iron, manganese, copper, and zinc are needed in very small quantities (Ahanger et al., 2016).

Macronutrients

Nitrogen plays a structural role biologically combined with Hydrogen, Carbon, Oxygen, Sulphur, and Phosphorus to create amino acids that are the building blocks of proteins (Khairnar & Nair, 2019). Nitrogen is responsible to play several roles in biochemistry of plant, including a major component of enzymes, nucleic acids, cell walls, proteins storage, chlorophyll, and a large variety of other cells (Moreira et al., 2015). Nitrogen also serves as a main role in agriculture by increasing crop production and enhancing food quality (Khairnar & Nair, 2019). Phosphorus (P) is a major plant growth and productivity nutrient in addition to nitrogen (N). It encourages seed germination, stalk and stem strength, root growth, flower and seed development, crop growth and yield throughout the plant. It also provides energy to drive different endergonic cell processes (Silitonga et al., 2018).

Besides, Potassium (K) plays a role in plant development, including the root system (Moreira et al., 2015). K responsible for enzyme initiation, protein synthesis, photosynthesis, cation-anion balance, plant stress resistance, energy transfer, phloem transport, osmoregulation, and stomatal motion (Ahanger et al., 2016). In plant tissue, calcium present as a Ca^{2+} in which it is needed as a structural constituent in the cell wall and membranes since a high concentration has been observed in the cell wall (Moreira et al., 2015). In addition, it acts as a counteracts cation for inorganic and organic vacuole anions, and acts as an intracellular cytosol messengers(White &Broadley,2003). Calcium involves in cell expansion where the necessary amount of calcium has been demonstrated to promote root growth. Ca levels necessary for critical growth functions, however it is too low that they can approach those of micronutrients (Hasanuzzaman et al., 2018). These 3 macronutrients (nitrogen, phosphorus, and potassium - N, P, K) can be supplied through an NPK fertilizer. While for calcium is derived primarily from a liming agent such as limestone.

Fertilizer

Fertilizer is a chemical agent that stimulate growth of plants and fruits. Fertilizer are usually applied either through the soil (for roots system) or through foliar feeding (uptake through the leaves) (Rai et al., 2015). Using fertilizer is one way of providing all nutrients the plant requires (kulkarni & Goswami, 2019). It is anticipated that NPK fertilizer will promote field application and raise the nutrient content in the soil (Silitonga et al., 2018). In addition, farmers nowadays continue to use this type of inorganic fertilizer as it can provide fast nutrition. However, the cost of production rises dramatically because it is expensive. Research has also shown that using excess fertilizer to support plant growth gives more drawbacks than benefits.

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Eggshells

Chicken eggshells, an extra-cellularly assembled bio-ceramic composite of calcium, have been used to conserve egg content and provide the calcium needed to form the chicks' skeleton (Kristl et al., 2019), was found to be the most ideal substitute for limestone (Oliveira et al., 2013). Most of the literature research indicates that the shell contains 94-97% of calcium carbonate, $CaCO_3$, with some sources providing up to 98.2% higher values and as low as 89.5%, leaving eggshells a valuable natural source calcium (Kristl et al., 2019). Besides, eggshell contains proteins (organic matter) as important constituents with minor quantities of lipids and carbohydrates (Mittal et al., 2016). Calcium from eggshell waste could be used for the effective extraction of such heavy metal ions, such as cadmium, lead and copper (Francis & Abdel Rahman, 2016). Minakshi et al. (2018) state that the high calcium content in the eggshells can be used to store energy. The application of eggshells as a strong solid catalyst for converting vegetable oil through transesterification process with methanol into methyl esters was also evaluated in biodiesel production (Oliveira et al., 2013; Faridi & Arabhosseini, 2018). Other than that, eggshells can be used as a coating substance like inkjet or CO_2 sorbent coating pigments, as well as heavy metal immobilization compost (Nasrollahzadeh et al., 2016). Cree & Rutter (2015) state that eggshell meal in Canada is an acceptable source of calcium for crops and minerals used as feed for poultry, dairy cattle, pigs, and pets. Eggshells also contains a percent of collagen, biomaterials used in either cosmetic or medical care of skin grafts, plastic, dental therapy, and pharmaceutical operations (Faridi & Arabhosseini, 2018).

Despite all these functions and valuable chemical components, eggshells have not yet received adequate attention to turn them from waste into new materials (Oliveira et al., 2013). Most eggshell waste is cumulated on-site without pre-treatment (Faridi & Arabhosseini, 2018). Though not dangerous, the issue of environmental contamination can still occur, as the biological protein matrix attracts rats and worms and causes public health problems. Recently, as the field of Research and Development (R&D) has enhanced and the technology has updated, the study of these eggshells' benefits has been widened. Eggshells are the most effective alternative to calcareous as they are biodegradable and plentiful (Francis & Abdel Rahman, 2016). The eggshell contains a purified mineral calcite, a more balanced form of $CaCO_3$ than carved calcite, which contains impurities including clay, sand, and other minerals (Cree & Rutter, 2015).

Using eggshells as a liming agent give rise to many advantages. Using eggshells enhances the biological fixation of N_2 in acid soils and increases the net organic nitrogen mineralization (A. Moreira et al., 2015; Nanda K. Fageria & Nascente, 2014). In addition, the production of field crops improves due to increase of soil quality in Ca and Mg and reduced soil acidity (Moreira et al., 2015). However, the use of lime is differed for every plant. The amount of lime needed depends on the type of soil, the quality of the liming materials, the cost and species of crops or cultivars.

Soilless Medium Growth

Soil quality at a particular plantation site plays an important role in deciding whether the sites can promote plant growth. Soil quality could be reduced when compaction, salt accumulation, excess nutrients and chemical substances and harmful substances present (Schloter et al., 2003). The application of excess fertilizers, those which provide nitrogen, are often accused of creating acidic soil when the materials containing ammonium are converted into nitrate (Caires et al., 2016). However, the practise of soil acidity correction needs to use a particular type of

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compound such as limestone. The use of limestone can have a further negative impact on the environment as they are non-renewable deposits (Oliveira et al., 2013). To alleviate all these issues, a soilless medium is applied to create a better version of a cultured medium.

Cocopeat

Effective growing media or substrates are important for producing quality horticultural crops. Due to the less effective and limitations contains inside soil media, planting using soilless method is preferable. Soilless production systems requiring materials other than soil are deemed necessary for the global cultivation of horticultural crops. The systems can increase productivity while improving sustainability through reusing drainage water and fertilizers (Ketter et al., 2015). Coconut coir peat (CP), a natural renewable energy source that can act as a soilless growing media, has been regarded as an ecologically substitute for peat (H.K.M. Kumarasinghe, S.S. Subasinghe, 2015). Cocopeat is an agriculture coproduct made of coconut peel after coconut fibre extraction from the coconut husk (Behavior & People, 2007; H.K.M. Kumarasinghe, S.S. Subasinghe, 2015). Ilahi & Ahmad, (2017) state that this coconut husk comprises a fibrous substance known as coir, which contains dense coconut fruit mesocarp. The long coir fibre extraction method leaves waste products, comprising of short coir fibre and dust, (Ilahi & Ahmad, 2017). Long fibres are used to produce brushes, seats, spinning systems and mattress filling, while short fibres (2 mm and less) and dust have been further split, compressed and washed for the production of a new product as a planting medium (cocopeat) (Alzrog, 2013).

As a cultivated medium, cocopeats could cultivate several good quality crop species throughout the tropics (Ilahi & Ahmad, 2017). Cocopeat is an ideal growing medium with suitable pH level, electrical properties, and other chemical characteristics. Indeed, cocopeat has good physical qualities, large pores, high content of water, low degree of decline, low density of bulk and gradual biodegradation (Behavior & People, 2007). The results of several studies have shown that cocopeat as a soil medium part or its alone is good for plantation of roses, potted plants, and even vegetables (Ketter et al., 2015). Cocopeat finds, use in seed germination, nursery raising, root cutting and other methods of plant propagation, plant hydroponic systems, plant cultivation, soil conditioning and so on (Marjenah, 2016). Research found that medium size between 3 mm to 0.5 mm particle is the best for greenhouse cultivation for varieties of vegetables including tomato, bell pepper, and cabbage (H.K.M. Kumarasinghe, S.S. Subasinghe, 2015).

Methodology

Biomass Sample

Raw eggshells were collected from housing area and cafe in UiTM Arau. The eggshells then washed with tap water and allowed to dry at open area. The eggshells then were oven to complete the drying process. The oven eggshells were kept in a container to avoid any contact with moisture. Peatmoss for germination and cocopeat as one of the components for medium treatment was bought near Kangar, Perlis. Fertilizer X and Y and red spinach seed were bought at Nursery in Kangar, Perlis. Three different mediums were observed in this study with each of them differing in ratios. The treatments are shown on the Table 1:

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Table 1: Different Ratio Of Growing Medium Treatment

Treatment	Medium	Percentage
T1	Cocopeat (control)	100
T2	Cocopeat + eggshells	50:5
T3	Cocopeat + eggshells	80:20

Materials And Chemicals

Some of the chemicals used are nitric acid (HNO_3) with purity of 65%, and hydrogen peroxide (H_2O_2) 30.0% purity. Materials used in this study includes eggshells, peatmoss, ocopeat, distilled water, deionized water, multiple standards, beaker, volumetric flask, measuring cylinder, pipette, weighing balance, weighing paper, spatula. Instruments used in this study are oven, Microwave digester, ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry), FTIR (Fourier Transform Infrared Spectroscopy).

Chemical Analysis

Four different analyses were conducted to determine the chemical characterization of cocopeat and eggshells as soilless growing medium using chemical analyses: moisture content, ICP-OES analysis, FTIR analysis, and pH analysis.

Moisture Content

Eggshells and cocopeat were weighed separately using weighing balance. The aluminium cup was made using aluminium foil and was first weighed before eggshells and cocopeat were put inside. Initial weighed of each sample was recorded. The samples then were oven dried at 105 °C overnight. The sample was allowed to cool first in the oven before the final weighed were measured. The samples were weighed along with the aluminium cup. The final sample weighed was recorded by subtracting the weighed of the aluminium cup.

ICP-OES Analysis

The Inductive couple plasma optical emission spectrometer (ICP-OES) was used to determine the inorganic matter content of the eggshells, cocopeat, Fertilizer X and Y. About 300 ± 1.0 mg of cocopeat and eggshells each with 3 samples were digested with 2.5 mL of 65% HNO_3 and 2.5 mL of 30% H_2O_2 in high performance microwave digestion system, Ethos One. The temperature was ramped to 90°C for 15 minutes, held for 5 minutes, then ramped again to 180°C for 10 minutes and held for 15 minutes. The digested samples were analyzed using Perkin Elmer Optima 8000 ICP-OES.

FTIR Analysis

The wavelength of the organic material inside cocopeat, eggshells, fertilizer X and Y were measured and identified using ATR-FTIR. Powder form of cocopeat and eggshells were placed on the sample holder using a spatula and run using a flat and wide tip. While the liquid form of fertilizer X and Y, the sample was placed on the sample holder using dropper and run by using ATR. The sample holder was cleaned using alcohol every time a new sample was loaded. The graph obtain from the FTIR analysis was smooth out first and the important peak was labelled automatically. The bond for the labelled peak was then identified.

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pH Analysis

pH analysis was carried out to differentiate medium acidity for each treatment. The pH paper was placed inside each medium, and the change of colour on the pH paper was observed.

Experimental Set Up

Three PVC pipe, 24 pots, and 48 screws were used to build the hygrowpipe. Ten holes was drilled on one side of the PVC pipe where each of these holes were placed with single pots except for one of them. The hole that did not fit with the pot was used to measure the water level inside the PVC. Its surface then was closed with tape to avoid any contamination and impurities. Each pot was screwed to maintain its placement, and each was labelled according to the treatments, replicate and individual. At the end of the PVC head, a small hole was made to insert a small pipe which connect the PVC pipe with fertilizer container. Once all the components were attached, the three different mediums were placed inside the pots following their label. The Hygrowpipe for observing plant performance was set up in an open system as schematically shown in Figure 1.

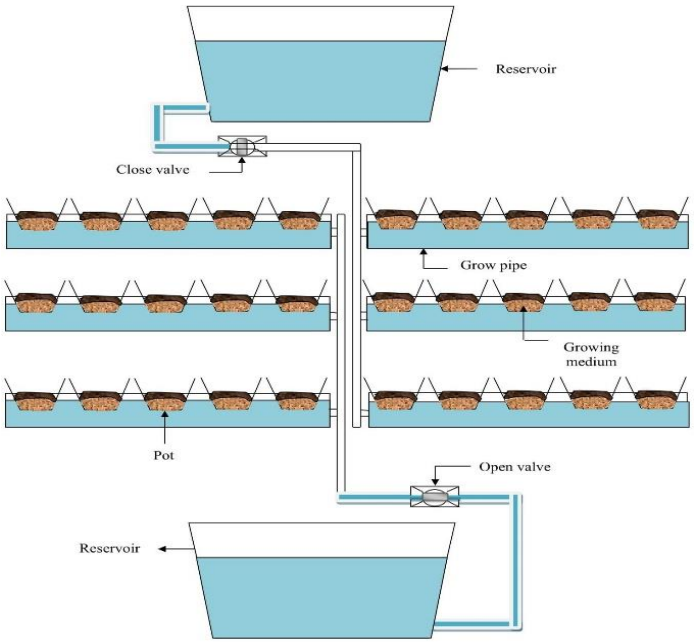

Figure 1: Shows The Schematic Diagram Of The Hygrowpipe Set Up

Experimental Design

This study used three different treatments: T1 is cocopeat solely which act as a control, T2 with 50% eggshells and 50% cocopeat and T3 with 20% eggshells and 80% cocopeat. Each of this treatment was replicated 4 times and each replication was divided into two individuals.

Plant Growth Performance

The seed of the red spinach was first germinated in the tray with peatmoss medium. After 7 days germination in the propagation area, the plants were moved into the medium treatment under an open area. Performance of the plant growth was measured a day after the plantation in the medium treatment. Heights and number of leaves were observed in each plant. The observation was from 22nd April until 27th May, where the data was recorded every 5 days.

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Results and Discussion

Moisture Content

Table 2 shows cocopeat has the highest moisture content with 98.04%, indicating that it can hold much water. This is because the cocopeat perlite mixture absorbs water over time, which results in a relatively high wettability of the mixture. Similar findings from other study stated that cocopeat has an extremely high-water retention capacity of 912.54%, which can be attributed to its low saturated hydraulic conductivity of 0.1 cm/s (Ilahi & Ahmad, 2017). Unlike eggshells, the shells' hard and solid characteristics only contain 1.96 % of moisture. This indicates that the eggshells neither able to retain nor absorb water as good as cocopeat.

Table 2: Moisture Content Of Cocopeat And Eggshells

Raw material	Moisture content (g)	Percentage (%)
Cocopeat	24.9819	98.04
Eggshelss	0.4986	1.96

ICP-OES Analysis

The efficacy of various treatments and fertilizers was determined using mineral content data obtained using inductive coupled plasma-optical emission spectrometry (ICP-OES). Since each nutrient contributed to the plant's growth, the nutrient's concentrations mainly influenced the plant's height, number of leaves, and the acidity of the treatment. Macronutrients are the most essential nutrients for plant, and the requirement for these nutrients in enhancing plants growth are strongly agreed (Ratmadanti, 2017). Figure 2 shows, Calcium (Ca) and Aluminium (Al) had the greatest average concentrations of the analyte. Calcium plays the significant role by acting as a protective barrier for plants from any illness. This is in agreement with similar finding by other researchers, stating that calcium supplementation may help minimize the severity of gray mold symptoms and postharvest Botrytis blight in rose blooms (Hua et al., 2015).

Aluminium (Al) concentration on the graph below is rather high. Although Al is not a necessary nutrient for plant growth, it can sometimes improve plant growth or generate other positive effects at low quantities. However, increasing the concentration raises the risk of Al toxicity in plant. Similar findings from other study stated that Al poisoning could disrupt root growth particularly in root-tips and lateral roots, where it becomes thick and turns brown (Rout et al., 2001). The finding however differs in this study. The plant growth under cocopeat alone resulted in healthy-growing red spinach with the highest height and number of leaves. The motive of this result is because Al toxicity only occurs when there is present of Al^{3+} in an acidic situation (Singh et al., 2017). Besides, each plant has a unique genotype, which means that they have varying tolerance levels for aluminum stress, which makes using cocopeat safe regardless of the high Al concentration.

Potassium (K) levels were discovered to be quiet high in cocopeat. K is one of the macronutrients that plays an important role in organs and turgordriven cells. It also activates cell metabolism, enzymes, photosynthesis, and protein synthesis. Plant growth therefore requires huge amounts of K^+ ions absorbed from the soil solution by roots and disseminated to the remainder of the plant. In most natural ecosystems, the availability of K^+ ions in the soil solution, which is slowly released by soil particles and clays, is frequently limiting for optimal growth (Rodríguez-vázquez et al., 2016)

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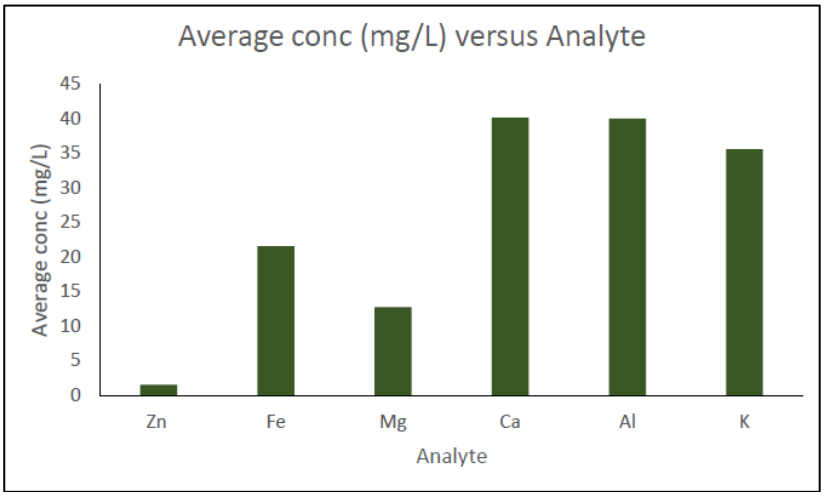

Figure 2: Composition Analyte In Cocopeat

Eggshell is a natural source of Ca and other essential minerals. Figure 3 shows eggshells contains other macronutrients like magnesium (Mg), and potassium (K). However, these nutrients value are very far from Ca. This is because previous studies demonstrate that K content reduced when Ca increases, and this occurred due to the antagonism of these two elements (Hua et al., 2015). A previous study by Litalien et al. found that when calcium and magnesium fertilizers are applied, the amount of K taken up by the plants decreases. Similarly, the intake of Ca and Mg decreases following excessive use of K. The usage of eggshells alone as a fertilizer can cause several health problems for the plant. Similar findings from another study state that potassium deficiencies lead to yellow searching or firing (chlorosis) (Meena et al., 2016). The fired margin of the leaf may fall out in severe situations of potassium insufficiency. Without sufficient potassium, the plants will have poorly developed roots, grow slowly and yield little seeds and cereals (Meena et al., 2016).

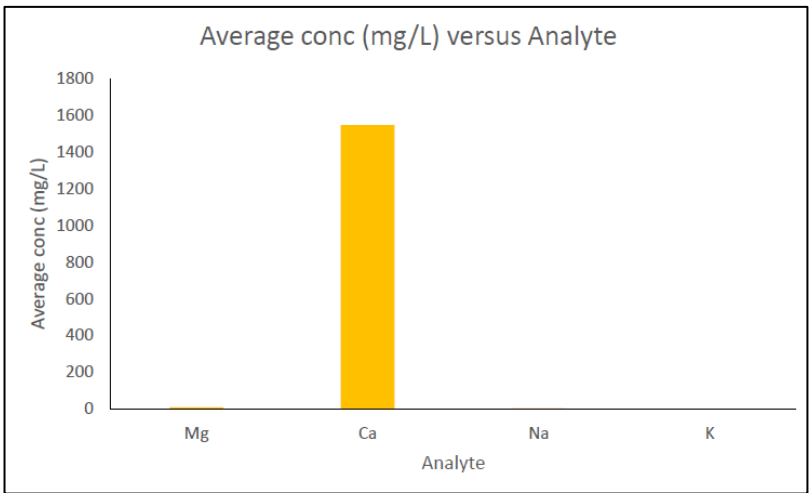

Figure 3: Composition Analyte In Eggshells

As referred to the Figure 4, It can be deduced that the composition of eggshells is comparable to Fertilizer X in that both contain a high concentration of Ca. This means that T2 and T3 may

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have extremely high Ca concentrations. However, an excessive concentration of Ca can have the opposite effect. As a result, the growth of the T2 and T3 plants is poor compared to T1. On the other hand, the analysis composition of fertilizer Y is considerably different. Fertilizer Y is rich in potassium and magnesium, but Ca is extremely low. As a result, plants in T1 develop exceptionally well since they have balanced nutrients when compared to other treatments.

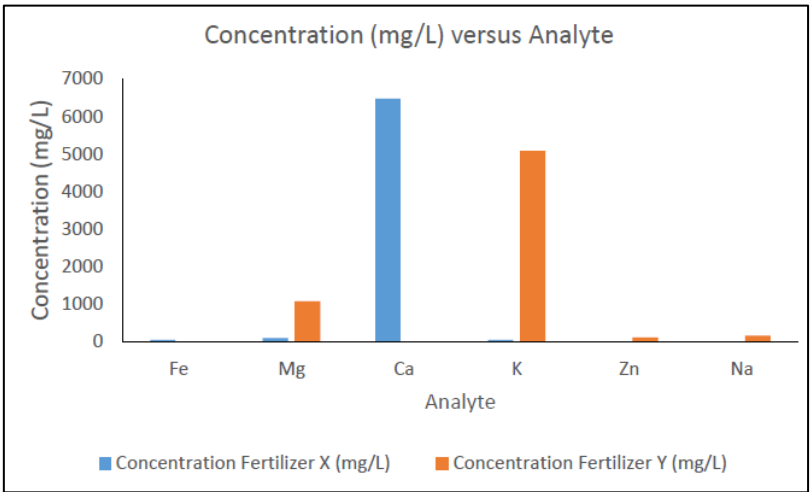

Figure 4: Composition Analyte in Fertilizer X and Y

FTIR Analysis

Figure 5 shows an overlap spectrum of fertilizer X and Y. The first three peaks for both X and Y fertilizer have the same spectrum. Peak at wavelength between 3400-3300 cm^{-1} correspond to the N–H stretching bands in aliphatic primary amine, while for 1648-1638 cm^{-1} wavelength peak indicate the presence of C=C stretching vibration of alkene. Peak at wavelength between 1420-1330 cm^{-1} indicate the presence of O-H vibration of alcohol. The remaining two peaks show the difference in the chemical bonds present. Fertilizer X's peak at a wavelength between 1050-1040 cm−1 indicates the presence of anhydride's CO-O-CO stretching vibrations. 1124-1087 cm^{-1} wavelength peak indicate the C-O stretching band of secondary alcohol in the Fertilizer Y. The remaining spectrum are C=C and C-H, indicating functional alkene and aromatic compound groups.

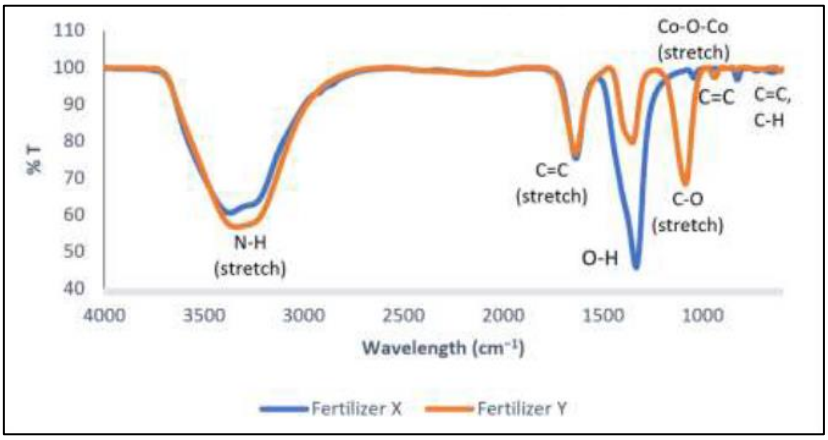

Figure 5: FTIR Spectrum of Fertilizer X and Y

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Each fertilizer shows the presence of oxygenated groups with a peak of O-H vibrations in both fertilizers. O-H vibrations between 1420-1330 cm^{-1} indicated the presence of phenol and alcohols in the fertilizers. The N-H stretching band from fertilizer X and Y are typically from ammonium nitrate compound which predominantly used in agriculture as a high-nitrogen fertilizer (Bakeer, 2016). The anhydride spectrum presence in fertilizer X comes from inorganic anhydrides: sulfur trioxide, SO_3 derived from sulfuric acid, and calcium oxide, CaO (Information, 2021).

Figure 6 shows the spectrum for the raw eggshells. The C-O vibration at the wavelength of 1440-1395 cm^{-1} indicates the presence of carbonate group. Peak at wavelength between 880-860 cm^{-1} correspond to the C-H bands in aromatic compound, while for 730-665 cm^{-1} wavelength peak indicate the presence of C=C vibration in alkene. The C-H bands band and C=C vibration of alkene come from the Protoporphyrin IX (PpIX), an immediate precursor of heme. The PpIX is the eggshells' main pigment, resulting in the shell's brown colouration (Li et al., 2013). C-O vibration indicates the presence of carbonate group which coming from calcium carbonate structure. Calcium carbonate nanoparticles are arranged into ordered crystals by protein, forming a calcite shell.

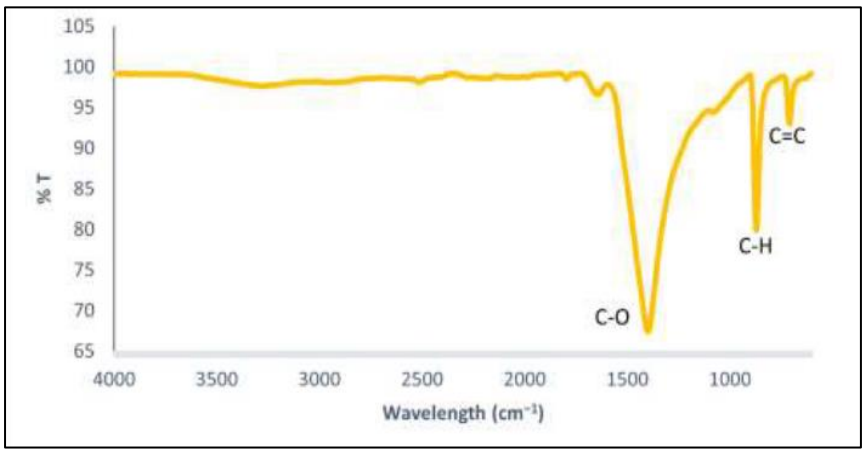

Figure 6: FTIR Spectrum of Eggshells

Cocopeat spectra obtained from the FTIR analysis contain a broad range of wavelengths. Figure 7 shows a peak for alcohol and carboxylic group at a wavelength of 3550-3200 cm^{-1}. The C=C stretching vibrations at 1650-1600 cm^{-1} indicates the presence of conjugated alkene, and the peak at wavelength between 1275-1200 cm^{-1} indicates the presence of C-O stretching vibration in alkyl aryl ether.

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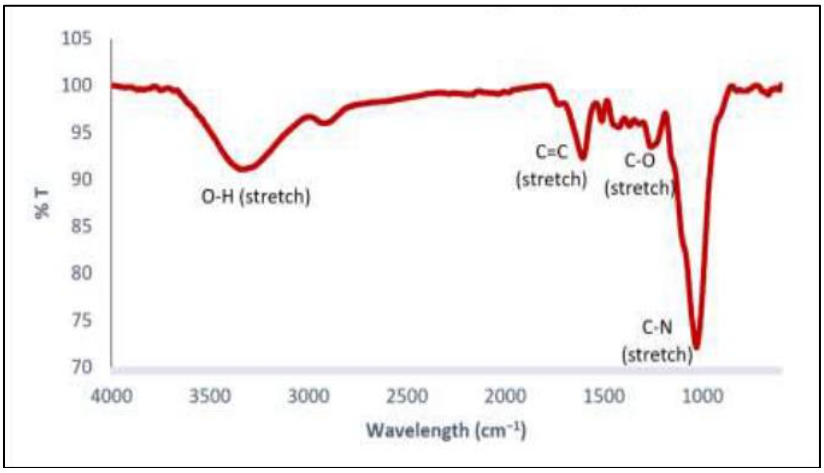

Figure 7: FTIR Spectrum of Cocopeat

The last peak shows a C-N stretching vibration of amine at the wavelength of 1250-1020 cm^{-1}. Cocopeat is known to be rich with nutrients as what have been mentioned before. An amine group appearing on the IR result shows that cocopeat contain ammonium nitrate compound known to have a high level of nitrogen. The C=C, C-O, and O-H bands come from the main components of cocopeat, which are cellulose, hemicellulose, and lignin.

pH Analysis

Soil pH has a vital influence in plants where it stimulates its soil properties and plants growth. The activity of microorganisms and the solubility and availability of nutrients are some of the most essential activities depending on pH (Gentili et al., 2018). The ideal soil pH balance for red spinach was found between 6 to 7. The pH balance, however, fluctuates based on the plant type, where certain plants require either above or below this range. Results indicate that T1 has a pH of 6 because cocopeat has a pH of 5.0-6.8, which is neutral to slightly acidic. T2 and T3 have a pH of 7 due to the inclusion of eggshells. The use of calcium oxide reduces acidity of the soil, releases exchangeable cations, and enhances soil fertility (Tongsiri et al., 2020).

Plant Growth Performance

Plant's Heights

Observing the plant's growth is crucial to determine the influence of the treatment being used. Figure 8 revealed that red spinach planted in T1 grows larger and healthier plant than in T2 and T3. T1 plant with heights of 29.49 cm prove that cocopeat medium is the best growing medium for red spinach planting. This finding clearly demonstrates that the ICP-OES nutrient values are legitimate. The presence of suitable macronutrients such as Ca and K in cocopeat increases red spinach's growth, resulting in taller plants. This is consistent with other researchers' findings that calcium is responsible for enhancing cell growth and development by triggering the growth of roots (Weissert & Kehr, 2017). The clear difference of the treatments can be seen on 6th reading. When the plant reached its maturity and harvested stage, available nutrients boosted its development.

T2 medium treatment demonstrates the appearance of the least tall of spinach with a height of 18.4 cm. Through observation, the 50% eggshell-medium growth resulted in a small and thin plant. Upon closer inspection, it was discovered that the plant's stem was extremely fragile when exposed to high pressure wind. The crushed eggshells were believed to cause the plant's

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sluggish development as the shells' sharp edges tend to injure the plant's root. T2 also demonstrates that broken eggshells turn greenish due to the formation of algae when utilized as a media. Even though crushed eggshells are supposed to have a significant amount of calcium, excessive calcium is not beneficial to plants. Adding calcium from the cocopeat combined with the eggshells causes the root to shrink and prevents nutrients from being taken up by the remainder of the plant.

Compared to the T3 plant, red spinach's growth under this treatment displays a result in the middle of the scale with a height of 25.61 cm. The graph reveals that the height rate increases with the reducing percent of eggshells. The stems of the plants in T3 are not as large as those in T1, but they are much thicker than those in T2. It could be because the percentage of eggshells utilized was decreased. Eggshells in T3 are also exhibiting the growth of algae on them, which has the effect of impairing the functions of the eggshell in this treatment.

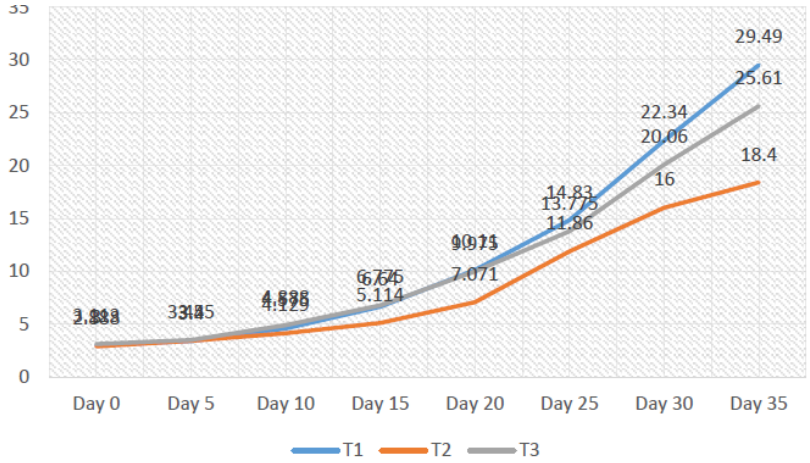

Figure 8: Comparison On Plants Height

Plant's Number Of Leaves

Figure 9 shows that the highest number of leaves was in T1 with 17.38 cm. This value is much higher when compared to T2 and T3 which are the same in plants height. The drastic different was observed on the 6th reading when the plant reach its maturity after 25 days of being planted in the medium. Highest concentration of K in the cocopeat enhance the build of numbers of leaves. This study finding is consistent with previous finding in which high K content improves photosynthesis capacity, reinforces cell tissue, and enables nitrate absorption (Weissert & Kehr, 2017).

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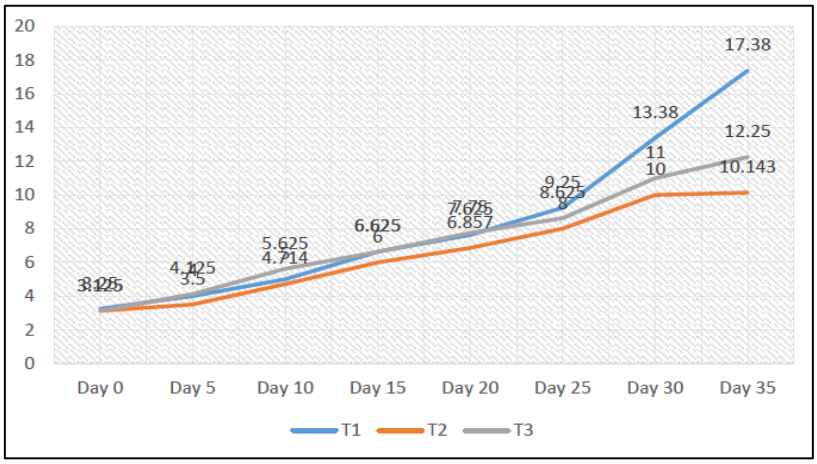

Figure 9: Comparison On Plants Number Of Leaves

T2 plants show the least number of leaves with only 10.143. Excessive concentration of Ca as what mentioned in the plant's height cause shrinkage of roots. The damage plant roots forbid the uptake of nutrients to the rest part of the plants. And due to that, the plants' produce small and short leaves along with a damaged visual appearance. On the 8th reading, there was noted that some of the leaves in T2 was death and shrink due to the least nutrient being transport to the leaves. This hence shows that by increased the Ca concentration does not really boost the growth of plant as what being stated in the past research.

T3 plant number of leaves are quite close to T3 value. The decrease amount of Ca somehow helps in increasing the number of leaves. Furthermore, the position of the plants influences the quantity of leaves produced. This is because if enough sunlight is available, the growth improves despite the presence of surplus nutrients. During the growing stage, insects, snails, and even parasites attacked most of the plant. Many holes were found on the leaves; some shrivelled and were lost due to infection. The leaves of the plants appear like they were scorched, and the infected leave can infect the neighbouring leaves.

Plant's Fresh Weight

Refer to Figure 10, Red spinach grown in T1 has the highest value in term of fresh weight compared to T2 and T3. The plants growth rate was mainly determined by fresh weight. As we can say from the ICP-OES results in cocopeat, it demonstrates that cocopeat has greater potassium concentrations, which is responsible for root development and is engaged in water regulation and transfer of plant reserve components (Weissert & Kehr, 2017). As the root developed significantly, the uptake of nutrients in the rest of the plant also increased, producing a well-growing plant. This is in good accordance with previous results from other researchers, which show that when the root weight grows, the length and height of the new leaf are increased accordingly (Ason et al., 2015).

However, using fresh weight to determine growth rate is not well supported. This is because, plants have a high composition of water and the quantity of water in a plant will depend on the amount of water in its environment which is very difficult to control. This discovery is also backed by other research as it claims that weighing process of fresh plant may be affected by several environmental and technical aspects including relative humidity, air currents, and laboratory temperature (Huang et al., 2016). As a result, it is recommended that plant growth

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promotion tests be conducted using dry weight determination rather than fresh weight determination.

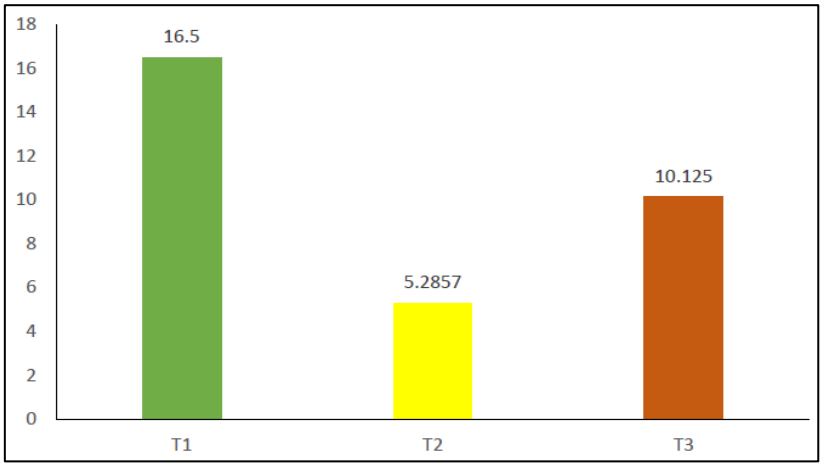

Figure 10: Comparison On Plants Fresh Weight

Statistical Analysis

In these results, the null hypothesis for the three analyses can be rejected and conclude that some of the analysis have different means. Table 3 shows the p-value value obtain for heights, leaves, and weight are 0.008, 0.000, and 0019, respectively which are less than the significance level of 0.05. This show that the difference in treatment for the three analyses are practically significant.

Table 3: p-value of Plants Height, Number Of Leaves And Fresh Weight

Parameter	Sum of Squares	df	Mean Square	F	Sig.
Heights	759.276	2	379.638	6.128	0.008
No. of leaves	500.333	2	250.167	11.967	0.000
Plant weight	565.083	2	282.542	4.845	0.019

Conclusion

In this study, the influence of different percent of eggshells in a growing medium is observed on the growth performance of red spinach (*Amaranthus dubius*). Eggshells is known for its high calcium content and due to its abundance, this poultry waste was suggested as and organic fertilizer by many researchers. Throughout this study, the growth of red spinach on 50% eggshells medium was found to be poor. Elevated amount of calcium shrinks the roots and restricts transporting nutrients to other regions of the plant. Red spinach with cocopeat treatment demonstrates great growth performance. With sufficient nutrients from cocopeat and enough nutrients from the fertilizer, the plant can grow to the height of 48 cm with 17 leaves. As a result, this is the first investigation on plant growth performance in the presence of eggshells in a Hygrowpipe.

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