



Influence of Palm Oil Mill Effluent (POME) on growth and yield performance of Brazilian spinach (*Alternanthera sissoo*)

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ABSTRACT

The presence of abundant oil palm residues in Malaysia prompted the need to utilize this waste to avoid environmental pollution. Palm oil mill effluent (POME) is a thick, brownish liquid effluent comprising large amounts of solids and high organic content, convertible into a valuable source of biomass. Based on the nutrient content of POME, this waste has the potential to be utilised as an alternative source of plant nutrients and organic medium in different agricultural crop production. Very recent Brazilian spinach (*Alternanthera sissoo*) is getting rapid interest among scientists and nutritionists for its easy growing and great nutritional values. But growing this spinach in Malaysia is still unfamiliar and no information about growing it using POME. Therefore, a study was conducted to determine the effects of different ratios of POME on growth and yield performance of Brazilian spinach (*Alternanthera sissoo*). The experiment was conducted at Faculty of Sustainable Agriculture, Universiti Malaysia Sabah, Sandakan campus, Sabah, Malaysia. The stem cuttings of Brazilian spinach were transplanted into polybags containing different ratios of POME, cocopeat and sands as T1 (70% POME + 15% cocopeat + 15% sand), T2 (60% POME + 20% cocopeat + 20% sand), T3 (50% POME + 25% cocopeat + 25% sand) and T4 (normal soils) as control treatment following randomized complete block design (RCBD) with four replications. Among all the measured parameters significantly ($P \leq 0.05$) the highest plant height (30.68 cm), maximum numbers of branches (14.50), maximum numbers of leaves (60.50), canopy coverage area (29.13 cm²), highest growth rate (78.0%), maximum fresh weight (330 g) and maximum dry weight (79.63 g) all were achieved from the Brazilian spinach grown under T1, followed by T2 and T3. So, from the overall findings undoubtedly it can be concluded that T1 was the best treatment for overall growth and yield of Brazilian spinach.

Keywords: Brazilian spinach, POME, growth, yield, biomass, organic manure

INTRODUCTION

Brazilian spinach (*Alternanthera sissoo*) is an extremely nutrient-rich leafy vegetable plant species native to Brazil and South America. It packs high amounts of carotenoids, vitamin C, vitamin K, folic acid, iron, and calcium. Another most important matter of consideration is of growing Brazilian spinach is very easy and convenient. This plant belongs to the Amaranthaceae family, which is now popularly growing in Malaysia. This vegetable is also known as the "Poor Man's Spinach", Sissoo spinach, Sambu and Samba lettuce. Brazilian spinach leafy vegetable is a low-growing perennial that forms a neat stack of up to 30 cm high, rather than spread out on the mat. This growth habit makes it a handy plant for edging paths, especially in partial shade because it is quite shade tolerant (Bassingthwaighe, 2018). The leaves are medium green, round and crooked. The flowers are tiny, green, and white. It is suitable only for subtropical and tropical areas. Although it prefers a moderate to rich loam, it does not like waterlogging. Plant is tolerate in full sun to medium shade. This soft plant can grow up to 30 cm tall with leaves about 2.0-3.5 cm wide. Growing media that is used for growing plants is one of the basic components are critical to initiate a culture. Among different types of growing media cocopeat and sands are commonly used in organic production. Cocopeat is a natural substance produced by the extraction of coconut husk, it rots easily and pest and weed-free material.

Brazilian spinach used to require a minimum amount of fertilizer application as they are very quick growing crops. However, to get a good yields proper fertilizer management is important. Besides that, the use of fertilizers is important to improve soil fertility in order to cater for plant growth from the early stages of cultivation to harvest. The use of synthetic fertilizers today has become more popular over organic fertilizers. However, the demand for organic fertilizer is also gaining more popularity among vegetable gardeners globally, where mainly cattle and poultry manures and plant-based compost are very common. Alternatively, in Oil Palm growing countries like Malaysia one of the organic fertilizer types has been produced from Palm Oil Mill Effluent (POME), which is expected to compete with other organic fertilizers in the market in the near future. The processed POME can be used as an organic fertilizer that is easily found in any oil palm industries at the Sabah state of Malaysia.

POME contains 95-96% water, 0.6-0.7% oil and 4-5% fat. POME liquid chocolate and acidic as pH 4-5. POME is a colloidal suspension originating from a mixture of sterilizer condensate, separator sludge and hydro cyclone wastewater in a ratio of 9:15:1 respectively (Wu et al., 2010). Nutrients contain in POME are nitrogen, phosphorus, potassium, magnesium and calcium, which are all vital nutrients elements for plant growth (Habib et al., 1997; Muhrizal et al., 2006). The potential for using POME as a cheap organic fertilizer that may offer an alternative to the excessive application of chemical fertilizers (Wu et al., 2009). Proper use and safe disposal of POME in the land environment would lead to improved soil fertility and contribute to environmental sustainability (Oviasogie & Aghimien, 2003). Their results showed enrichment of soils with regards to phosphorus, nitrogen, calcium, magnesium, sodium and potassium following the application of the POME. Copper, iron and lead were said to be predominant in their organic forms. At the same time, zinc was particularly present in its exchangeable form that biologically treated POME has been widely used in the oil palm plantations for irrigation purposes and can be employed as a liquid fertilizer (Wu et al., 2009). The increased growth and yield performance of several crops have already been reported worldwide by the application well decomposed and treated POME. Kanakaraju et al. (2016) stated that an increased application of POME sludge increased the growth and yield of water spinach (*Ipomoea reptans* Poir). Furthermore, augmented maize biomass and yield have been reported using POME sludge as an amendment by Mohd Nizar et al. (2018). On the other hand, plant growth and yield of tomato was increased significantly with increasing POME effluent treatments (Maliki et al., 2020). Based on the above findings we hypothesized that growth and yield of Brazilian spinach might be increased by the application of POME as an amendments with other commonly used growing media.

However, the continued use of synthetic fertilizer usually reduce soil productivity, hamper the activity of beneficial soil microbes and may cause various diseases to consumers. To protect the environment and to reduce the risk of human health, POME organic fertilizer was used to replace the use of synthetic fertilizers. On the other hand, Brazilian spinach have been chosen in this experiment because of its criteria introduced as one of the easy, fast-growing and high-value commercial vegetable crops in Malaysia. So, in this experiment the

effects of different ratios of POME on growth and yield performance of Brazilian spinach were compared and the best combination was recommended for the better growth and yield of Brazilian spinach.

MATERIALS AND METHODS

Study location, treatments and experimental design

This study was conducted at rain shelter 10, Faculty of Sustainable Agriculture, Universiti Malaysia Sabah, Sandakan campus, Sabah, Malaysia from January 2020 to December 2020 located at 5.9297° N latitude and 118.0107° E longitude. The average prevailing temperature into rain shelter ranges in between 25 °C - 35 °C. In this experiment a combination of various ratios of POME, cocopeat and sands were used to prepare the growing media as treatment. There were 4 different treatments including control are presented in Table 1. In this experiment, no soils were used with T1, T2, and T3 to avoid soil-borne diseases. The prepared media of every treatments were filled into polybags (18 cm × 15 cm) with four replications and arranged in randomized complete block design (RCBD).

Table 1. Treatment combinations of POME, cocopeat and sands used in this study

Treatments	Media combinations
T1	70% POME + 15% cocopeat + 15% sand
T2	60% POME + 20% cocopeat + 20% sand
T3	50% POME + 25% cocopeat + 25% sand
T4 (Control)	Normal farm soils

Preparation of cutting and transplanting

High quality, healthy (insect and disease free) and freshly harvested Brazilian spinach stem cuttings were obtained from nearby nursery. After that, about 6 - 8 cm long stem cuttings with 3 - 4 nodes were prepared using a sharp knife or secateurs from the collected stems. Excess leaves were removed from the prepared cuttings to reduce high transpiration rate or quick drying. Finally, well prepared Brazilian spinach stem cuttings were transplanted into every polybags containing the media mixtures of T1, T2, T3 and T4, respectively with 4 replications. Four cuttings were transplanted into each polybag. All leaves from the bottom half of the stems were removed and buried half of their length into the media and watered immediately to keep moist and to maintain saturated condition.

Watering, weeding and pest management

Watering was done daily in the morning and evening. The frequency of irrigation was twice a day depending on weather conditions on the day. Approximately 200 ml of water was applied manually into each polybag as irrigation. Weeding was done manually if it grows around Brazilian spinach and polybags. Weeds were removed immediately after emergence without giving any chance to interfere with nutrients, lights, and spaces and to avoid it becoming a host to pests and diseases. Insect pests like aphids was seen sometimes and manually killed and also sprayed bio pesticide (Bio - Attack Organic Pesticide / Racun Serangga Organik) as soon as possible because it can lead to spread diseases that may cause failure to produce healthy Brazilian spinach.

Data collections

Data on Brazilian spinach morphological growth and yields which included plant height (cm), numbers of branches, numbers of leaves, canopy coverage area (cm²), growth rate (%), plants fresh weight (g), and plants dry weight (g) were recorded. For every parameters data were collected 3 times and average was calculated.

Plant height (cm)

The height of the plant was measured starting from the emergence of a new shooting using a ruler. The height of the plant was measured from above the medium level up to the top shoot every week more or less after one week of transplanting of Brazilian spinach stem cuttings and initiation of new shooting from the cuttings.

Measurement was done using a measuring tape that is measured from the ground level to the end of the shoots of Brazilian spinach. The unit of the measurement was used as centimetres (cm). Data were taken from three different shoots of the same polybags and made the average to consider as data from one replication and continued to the next. The average height of the plants was taken every week until the final harvesting. The height of the plant was taken to see the growth performance of each plant by the application of different ratios of POME.

Numbers of branches and leaves

Numbers of branches were counted and recorded until the final harvesting of the plants and analysed. Numbers of leaves were counted three times a week until the final harvesting.

Canopy coverage area (cm²)

Apart from the height, the plant canopy coverage area (CCA) was also measured using a measuring tape. Canopy coverage area was analysed by collecting Brazilian spinach branching growth and coverage diameter for each polybag for all treatments and replications following the formula developed by Thorne et al. (2002) as $CCA = \frac{2}{3} \pi H (A/2)$. Here, H - branch height (m), A - coverage diameter (m) of each polybag.

Growth rate (%)

The relative growth rate (RGR) of Brazilian spinach was calculated by subtracting the second measurement of plant height from the first measurement of plant height and dividing the value by the number of days between the two measures and expressed in percentage (%).

Plants fresh weight (g)

For yield parameters, harvested fresh weight as well as the dry weight of Brazilian spinach were recorded. Treatment wise fresh weights of Brazilian spinach were measured after the final harvest from every replications and the average weight was calculated from the recorded data.

Plants dry weight (g)

After weighing the fresh weight of Brazilian spinach, the samples were kept in an electric oven at 70 °C for 72 hours until the constant dry weight was achieved. Finally the average dry weight was measured from each treatments and replications. Significant variations were found for most of the measured parameters under different treatments.

Statistical Analysis

Recorded data were subjected to one-way analysis of variance (ANOVA) using Statistical Analytic Software (SAS) Version 9.4 software. When F was significant at the $P \leq 0.05$ level, treatment means were compared and separated using the least significant difference test (LSD).

RESULTS AND DISCUSSION

Effect of POME on Brazilian spinach growth and yield

Throughout the study period parameter wise data were collected and recorded every week to see the differences that occurred in Brazilian spinach plants by the application of different ratios of POME organic manures. Actually this study involved the use of a mixture of different media, namely cocopeat, sand, and palm oil mill effluent (POME), from which a well-balanced media can be prepared to determine the most effective different ratios of POME for the production of Brazilian spinach and other crops as well. Media culture has an important role in maintaining the moisture around the roots, channelling oxygen and sufficient nutrients to plants (Salleh, 2016). All of the media used in this study is a mixture of organic materials that can also replace the use of chemical fertilizers and to reduce environmental pollution. Moreover, waste from agricultural production can also be used to the maximum.

Growth and yield of Brazilian Spinach

Plant height (cm)

Plant height was found to differ significantly ($P \leq 0.05$) over control treatment after application of different ratios of POME manures (Table 2, Figure 1). The highest plant height (30.68 cm) was measured under T1, which was statistically similar with T2 (30.38 cm), followed by T3 (28.48 cm) and the lowest was found under the control treatment T4 (27.40 cm). From the results it was observed that 11.97% increase of plant height under T1; 10.88% increase under T2 and 3.94% increase under T3 over the control treatment T4, respectively (Table 2, Figure 1). This increase of plant height surely due to the maximum use of POME manures compared to the cocopeat, sand and only soils. Rosenani et al. (2016), conducted an experiment to observe the growth performance of Oil Palm seedling with induced application of POME compost and reported an increase of plant height over control treatment. Similar findings were also reported by Nizar et al. (2018) in case of increased maize plant height grown in different types of POME sludges compared to control treatment. A significant increase of kangkong (*Ipomoea aquatica*) plant height have been report by Loh et al. (2019) with the application of POME supplemented with chicken manure. Tomato plant height was reported to increase significantly in a mixed soil media amended with palm oil mill effluent and rubber processing effluents (Maliki et al., 2020).

Numbers of branches

For the numbers of branches there were no significant variations among three treatments of POME application (Table 2, Figure 1). The highest numbers of branches (14.50) were produced by those plants grown under T1 and T2, followed by T3 (14.00) and significantly ($P \leq 0.05$) the lowest numbers of branches (13.00) were counted in the plants grown under control treatment T4, respectively (Table 2, Fig. 1). From the findings it was clearly observed that highest proportion of POME induced the plants to grow more braches over the control treatment. This finding is in accordance with the findings of Falodun et al. (2011), who found that the numbers of branches in soybean plants increased significantly after application of 5 t/ha and 10 t/ha of POME as organic manures compared to control treatment. May the POME is a source of plant growth-promoting substances and nutrient elements which stimulate the branching and increase the leaf numbers (Gandahi & Hanafi, 2014; Mahmud & Chong, 2021).

Numbers of leaves

Production of numbers of leaves by Brazilian spinach was significantly ($P \leq 0.05$) affected by the application of different ratios of POME manures (Table 2 and Fig. 1). The highest numbers of leaves (60.50) was counted under T1 with the highest ratios of POME (70% POME + 15% cocopeat + 15% sands), followed by T2 (59.25), T3 (58.5) and the lowest numbers of leaves (58.0) were counted under T4 (control treatment), respectively. But numbers of leaves under T1 and T2 were statistically similar ($P \geq 0.05$), while numbers of leaves under T3 and T4 were also statistically non-significant ($P \geq 0.05$). The results from the study proved that application of highest ratios of POME exhibited the highest production of numbers of leaves, which were found to goes down with decrease of POME ratios and finally the treatment with the lowest POME ratios produced the similar numbers of leaves with control treatment. This finding is accordance with the results described by Falodun et al. (2011), who conducted an experiment to observe the growth performance of soybean using different doses of POME. Loh et al. (2019) reported the increased numbers of leaves of Palm oil seedlings by the application of palm oil mill effluent (POME) supplemented with chicken manure. Furthermore, Malilki et al. (2020) opined that numbers of leaves of tomatoes increased significantly grown in soils amended with palm oil mill effluent and rubber processing effluents. Gandahi & Hanafi (2014) reported that POME may contain plant growth-promoting substances and nutrient elements which stimulate the branching and increase the leaf numbers.

Canopy coverage area (cm²)

Based on the results of the study presented in Table 2 and Fig. 1, different rations of POME application exhibited a mixed reaction on canopy coverage area of Brazilian spinach over control treatment. Significantly the highest canopy coverage area (29.13 cm²) was measured under treatment T1 with the highest concentration of POME, but interestingly the canopy coverage area tended to decrease at T2 (25.58 cm²) and T3 (22.42 cm²), respectively with the decrease of POME added ratios even reached to the lowest than that of the canopy coverage area under control treatment (28.01 cm²). Actually the canopy coverage area of Brazilian spinach under

T1 and T4 (control) were statistically similar ($P \geq 0.05$). May be the lower ratios of POME mixed with cocopeat and sands are not sufficient to provide required nutrients for increased canopy growth of the Brazilian spinach. Faridah (2001) reported that not only POME but also mixture of other recycled agricultural waste could be more balanced to increase crop yields. This includes rice straw and husk, empty oil-palm fruit bunches (EFB), sawdust, animal droppings, palm oil mill effluent (POME) and other materials.

Table 2. Effect of different ratios of POME on growth and yield parameters of Brazilian spinach

Treatments	Plant height (cm)	No. of branches	Numbers of leaves	Canopy coverage area (cm ²)	RGR (%)
T1	30.68 ^a	14.50 ^a	60.5 ^a	29.13 ^a	78.0 ^a
T2	30.38 ^a	14.50 ^a	59.25 ^{ab}	25.58 ^b	65.0 ^b
T3	28.48 ^{ab}	14.00 ^{ab}	58.5 ^b	22.43 ^c	58.0 ^c
T4 (control)	27.40 ^b	13.00 ^b	58.0 ^b	28.01 ^{ab}	63.0 ^b

Note; mean values with the same letter had no significant difference at $p < 0.05$; T1 (70% POME + 15% cocopeat + 15% sands), T2 (60% POME + 20% cocopeat + 20% sands), T3 (50% POME + 25% cocopeat + 25% sands), T4 (normal soils only), RGR – Relative Growth Rate.



Fig. 1. Growth of Brazilian spinach at week 6

Growth rate (%)

Plant relative growth (RGR) analysis refers to a set of concepts and equations by which changes in size of plants over time can be summarised and dissected in component variables. It is often applied in the analysis of growth of individual plants, but can also be used in a situation where crop growth is followed over time. Plant growth analysis is required to explain the differences in plant growth in terms of differences between species growing under same environmental condition or differences within a species growing in different environments (Pandey et al., 2017). Though the plant growth rate is depends on multiple variables but in this experiment the growth rate of Brazilian spinach was mainly measured based on changes of plant height in every 10 days interval until final harvest and expressed in percentage. From the results presented in Table 2 and Figure 1 a significant variation was observed for Brazilian spinach growth rate among treatments over control. Significantly ($P \leq 0.05$) the highest growth rate (78.0%) was counted under T1 (70% POME + 15% cocopeat + 15% sands) followed by T2 (65%), T4 (control; 63%), respectively and the lowest was seen under T3 (58%). This finding is sharply representing that addition of increased percentage of POME manure significantly increased the growth rate of Brazilian spinach. Similar findings has been described by Nizar et al. (2018) in maize plant growth rate who used treated palm oil mill effluent sludge as growing media. Furthermore, a significant increase of tomato plant

growth rate has also been reported by Maliki et al. (2020) by the application of soils amended with palm oil mill effluent and rubber processing effluents. Mahmud & Chong (2021) opined that POME may contain plant growth-promoting substances and nutrient elements which stimulate plant growth and health.

Plant fresh weight (g)

Fresh weight of any crop plant is very important as it is the ultimate consumable produce which assure the yield of the crops as well. As Brazilian spinach is an herbaceous and succulent leafy vegetable plants so the fresh weight was much higher over dry weight. From the overall findings it was observed that fresh weight of Brazilian spinach continued to increase with the application of increased ratios of POME. The highest fresh weight (330 g) of Brazilian spinach was recorded under T1 with the highest ratios of POME (70% POME + 15% cocopeat + 15% sands), followed by T2 (304.25 g; 60% POME + 20% cocopeat + 20% sands), T4 (291.0 g; control treatment) and the lowest fresh weight (289.50 g) was measured under treatment T3 with the lowest ratios of POME (50% POME + 25% cocopeat + 25% sands), respectively (Figure 2). The fresh weight under T1, T2 and T3 were statistically significant ($P \leq 0.05$), while the fresh weight of T3 and T4 were statistically similar ($P \geq 0.05$). The increased fresh biomass production in Palm oil seedling has been reported by Loh et al. (2019) by the application of different doses of oil mill effluent (POME) supplemented with chicken manure, which is in accordance with the findings of this study. On the other hand, Haryani et al. (2019), opined that fresh weight production of Napier grass was increased significantly by the induced application of 5 metric tonnes of POME per hectare. Increased fresh biomass yield in Tomato has also been reported by Maliki et al. (2020) grown in soils amended with palm oil mill effluent and rubber processing effluents. Mahmud & Chong (2021) described that the POME may contain plant growth-promoting substances and nutrient elements which significantly stimulate plant growth and biomass yield.

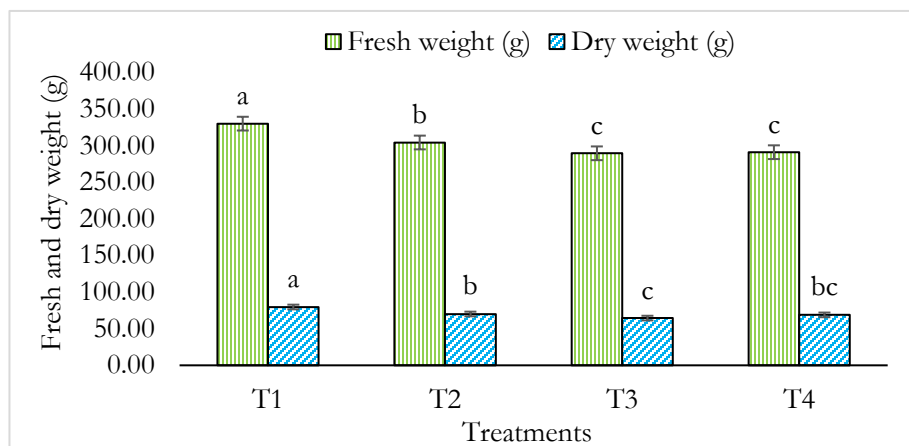


Fig. 2. Effect of different ratios of POME on fresh and dry weight of Brazilian spinach. Bar graph with different lower case letters are significantly different at $p \leq 0.05$; T1 (70% POME + 15% cocopeat + 15% sands), T2 (60% POME + 20% cocopeat + 20% sands), T3 (50% POME + 25% cocopeat + 25% sands), T4 (normal soils only).

Plants dry weight (g)

A significant amount of dry matter production by any plant is the expression of capability to survive or to adapt in any state of changes in biotic or abiotic environmental conditions (Raza et al., 2019). Changes of growing media or even application of varied types and doses of organic or inorganic manures also can influence the overall growth and yield of plants both negatively or positively. In this experiment a combination of different ratios of POME with other media (cocopeat and sands) was used to grow Brazilian spinach and the response from different treatments were also different, which were clearly observed from the significant variations of dry matter production among different treatments (Figure 2). From the final results it was observed that significantly ($P \leq 0.05$) the highest amount of dry matter (79.63 g) was produced by the Brazilian spinach grown under T1 with the highest ratios of POME (70% POME + 15% cocopeat + 15% sands), followed by T2 (70.08 g; 60% POME + 20% cocopeat + 20% sands) and T4 (68.85 g) under control treatment, while the lowest amount of

dry matter (64.50 g) was produced by the Brazilian spinach grown under the treatment T3 (50% POME + 25% cocopeat + 25% sands) with the lowest ratios of POME (Figure 2). Meaning is that the dry matter production increased with the increased application of POME similarly the dry production decreased with the reduced application of POME and the lowest was measured under the lowest ratios of POME application even lower than the control treatment. The dry matter production under T1, T2 and T3 differed significantly ($P \leq 0.05$), but T3 and T4 were statistically non-significant ($P \geq 0.05$). These results are in accordance with the findings of dry matter production in Soybean (*Glycine max*) reported by Falodun et al. (2011) with the application of POME and NPK fertilizers. Increased dry matter production in Maize (*Zea mays*) has also been reported by Nizar et al. (2018) grown in media mixed with treated palm oil mill effluent sludge. Loh et al. (2019) conducted an experiment to observe the growth and yield of Kangkong (*Ipomoea aquatica*) using palm oil mill effluent (POME) supplemented with chicken manure and opined a significant increase of dry matter production over control treatment. Furthermore, Maliki et al. (2020) described that dry matter production in tomato seedlings was increased grown in soils amended with palm oil mill effluent and rubber processing effluents. POME may contain plant growth-promoting substances and nutrient elements which significantly stimulate plant growth and biomass yield (Gandahi & Hanafi, 2014; Mahmud & Chong, 2021).

CONCLUSIONS

Throughout the study it has been proved that Brazilian spinach can be grown organically through a sustainable way of using POME as a cheap organic manure that may offer an alternative to the excessive application of chemical fertilizers. Among the three different ratios of POME with cocopeat and sands the highest growth and yield performance of Brazilian spinach was achieved under treatment T1 with the highest POME ratios (70% POME + 15% cocopeat + 15% sands). But the overall performance tends to decrease with the application of decreased ratios of POME over the control treatment. Among all the measured parameters the highest plant height (30.68 cm), maximum numbers of branches (14.50), maximum numbers of leaves (60.50), canopy coverage area (29.13 cm²), highest growth rate (78.0%), maximum fresh weight (330 g) and maximum dry weight (79.63 g) all were measured under T1, followed by T2 and T3. So, from the overall findings undoubtedly it can be concluded that T1 was the best treatment for overall growth and yield of Brazilian spinach, so T1 is recommended as the best treatment as well for the proper use and safe disposal of POME in the land environment which would lead to improved soil fertility and contribute to environmental sustainability.

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