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RESEARCH ARTICLE

EFFECT OF AZOLLA (*Azolla pinnata*) BASED ORGANIC FERTILIZER ENRICHED WITH EPPAWALA ROCK PHOSPHATE AND PADDY HUSK ASH ON THE GROWTH AND YIELD OF CHILLI (*Capsicum annuum) MICHHY01*)

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ABSTRACT

Excessive use of chemical fertilizers in agriculture has raised serious environmental and health concerns. In response to the challenges, there is growing interest in sustainable agricultural practices, particularly the use of organic fertilizers. This study was conducted to evaluate the effect of organic fertilizer derived from dried Azolla, blended with Paddy husk ash (PHA) and Eppawala rock phosphate (ERP), on the growth and yield performance of the green chilli variety *MICHHY01.* The experiment was conducted as a pot trial at the Faculty of Technology, University of Ruhuna using a completely randomized design with four treatments each replicated four times. The treatments consisted of varying ratios of Azolla, ERP, and PHA as follows: T1 (control - 0:3:3), T2 (3:3:3), T3 (6:3:3), and T4 (9:3:3). The basal growing media was prepared by mixing compost, sand, and coir dust at a 2:1:1 ratio (v/v). Growth parameters, including plant height, number of leaves, branches, leaf length, leaf width, and stem diameter, were measured at two-week intervals. Yield parameters, such as number of days to first flowering, number of flowers, number of pods, pod length, pod girth, and fresh weight of pods, were also recorded. The results revealed that the application of Azolla-based fertilizer at a 9:3:3 ratio (T4) significantly enhanced ($P<0.05$) both vegetative growth and yield performance. T4 exhibited the highest plant height (88.25 ± 1.65 cm), number of leaves (196 ± 4.35), and number of branches (82 ± 4) at the harvesting stage. Additionally, T4 recorded the minimum number of days to first flowering (60.00 ± 0.00 days), the highest number of flowers per plant (84.25 ± 6.30), and the greatest number of pods per plant (45.75 ± 3.52). Pod characteristics, such as pod length (12.375 ± 0.11 cm) and girth (10.360 ± 0.15 mm), were also significantly improved under T4. Furthermore, the highest total yield per plant (108.69 ± 5.19 g) was achieved in T4, demonstrating the effectiveness of the 9:3:3 Azolla-ERP-PHA combination in enhancing chilli production. Comparatively, the control treatment (T1), with a 0:3:3 ratio of Azolla:ERP:PHA, exhibited significantly lower vegetative growth and yield parameters. Therefore, the 9:3:3 combination of Azolla, ERP, and PHA showed considerable potential for enhancing chilli growth and yield under pot conditions; however, field validation and multi-season testing are required before recommending it for commercial cultivation.

Keywords: Azolla, Eppawala rock phosphate, Organic fertilizer, Paddy husk ash, Sustainability

INTRODUCTION

Agriculture is the mainstay of human civilization, providing food security and livelihoods for billions of people worldwide. However, the overuse of chemical fertilizers has led to significant environmental challenges, including soil degradation, water pollution, and greenhouse gas emissions (Thorat *et al*., 2022). In Sri Lanka, dependence on synthetic fertilizers is higher, resulting in increased soil salinity, heavy metal accumulation, and nitrate leaching, posing risks to both human health and the environment (Thorat *et al*., 2022). In recent years, the Sri Lankan government has prohibited the import of synthetic fertilizers that leading to a growing demand for organic fertilizers among farmers (Ruhunuge *et al*., 2021). This shift has emphasized the need for sustainable agricultural practices that rely on organic inputs to maintain soil fertility and crop productivity.

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Organic fertilizers, derived from natural sources such as animal manure, plant residues, and compost, offer a viable alternative to synthetic fertilizers. These fertilizers not only improve soil health but also reduce the environmental footprint of agriculture (Shaji *et al*., 2021). Among the various organic fertilizers, Azolla (*Azolla pinnata*), a small aquatic fern, has gained attention for its unique ability to fix atmospheric nitrogen through a symbiotic relationship with cyanobacteria (*Anabaena azollae*) (Setiawati *et al*., 2020). Azolla is rich in nitrogen, phosphorus, and potassium, making it an excellent biofertilizer for crop production (Setiawati *et al*., 2018). Additionally, Azolla can double its biomass every 3-5 days under optimal conditions, providing a rapid and sustainable source of nutrients for plants (Carrapiço, 2010).

Chilli (*Capsicum annuum*) is one of the most widely cultivated crops in Sri Lanka, with significant economic and culinary importance. According to the Department of Agriculture, Sri Lanka, the per capita consumption of dried chillies averages between 2.10 kg and 2.31 kg, with a significant amount consumed as green chillies (Dahanayake *et al*., 2012). The *MICHHY01* variety, the first local hybrid chilli recommended in 2015, is particularly valued for its high resistance to leaf blight and virus diseases (Dahanayake *et al*., 2012). The growth and yield of chilli are closely related to soil fertility and nutrient availability, making it an ideal candidate for exploring the potential benefits of organic fertilizers such as Azolla-based formulations.

Despite the promising attributes of Azolla, there is a noticeable gap in the scientific literature regarding its application and effectiveness in specific crop systems, particularly chilli cultivation. While Azolla has been widely studied in rice cultivation, its potential in other crops, such as chilli, remains underexplored (Setiawati *et al*., 2018). Furthermore, the combination of Azolla with other organic amendments, such as Eppawala rock phosphate (ERP) and Paddy husk ash (PHA), has not been extensively investigated. ERP is a locally available source of phosphorus, while PHA provides potassium, both of which are essential macronutrients for plant growth (Weerasooriya *et al*., 2020). The integration of these materials with Azolla could provide a balanced nutrient supply, enhancing soil fertility and crop productivity.
This study aims to address this gap by evaluating the effect of Azolla-based organic fertilizer, enriched with ERP and PHA, on the growth and yield of chilli (*Capsicum annuum*). Specially, the study will assess the influence of these formulations on vegetative growth parameters, soil nutrient availability, and yield performance. The findings are expected to provide valuable insights into the role of Azolla-based organic fertilizers in sustainable agriculture, contributing to the development of environmentally friendly and locally adaptable farming practices.

MATERIALS AND METHODS

Experimental procedure

The study was conducted at the Faculty of Technology, University of Ruhuna, Sri Lanka (6°03'51.8"N 80°32'36.1" E), located within the WL2a agroecological zone. This region is characterized by an average rainfall of 2500 mm, temperatures ranging from 22°C to 30°C, and an average relative humidity of around 80%.

The experiment was conducted as a pot trial using the *MICHHY01* green chilli variety (*Capsicum annuum*), a hybrid known for its high resistance to leaf blight and viral diseases. A completely randomized design (CRD) was employed, compromising four treatments with four replicates each, resulting in a total of 16 pots. Each pot served as an individual experimental unit. The polythene pots used for the trial had a height of 18 cm, a top diameter of 22.5 cm, and a base diameter of 16.5 cm. The growth medium was prepared by mixing compost, sand and coir dust in a 2:1:1 ratio. Each pot was uniformly filled with this medium and then subjected to gentle manual compaction.

The treatments consisted of varying ratios of *Azolla pinnata* (*A. pinnata)*, Eppawala rock phosphate (ERP), and Paddy husk ash (PHA), as follows: T1 (control - 0 Azolla:3 ERP:3 PHA), T2 (3:3:3), T3 (6:3:3), and T4 (9:3:3).

Determination of nutrient composition

Azolla was dried at 60°C for 8 hours. (Azhari *et al*., 2023) , ground into a fine powder and passed through the standard laboratory sieve to achieve a particle size less than 2mm before application. Rice husk ash was prepared by incinerating dry ri husk at 600 ^{o}C for 10-12 hours. (Mbakaan *et al*., 2014) in a muffle furnace. ERP was obtained from the local market.

The chemical analysis was performed on the individual raw materials, such as *A. pinnata* powder, ERP, and PHA. The physical properties of the fertilizer blend, including pH, electrical conductivity (EC), and moisture content, were determined according to the official method of the Association of Official Analytical Chemists (AOAC). Total nitrogen content was determined using the Kjeldahl method based on acid digestion, distillation and titration. Phosphorus content was determined by spectrophotometry, and potassium content by flame photometry, according to AOAC standard procedures (AOAC, 2016).

Crop establishment

Chilli seeds were sown in nursery trays filled with a 2:1 compost-to-coir dust mixture. After two weeks, seedlings were transplanted into pots containing a growing medium containing compost, sand, and coir dust in a 2:1:1 ratio. According to the manufacturer's analysis, the compost contained a primary nutrient composition of 1.0% Total Nitrogen, 0.5% P_2O_5 and 1.0% K_2O and before introducing Azolla, PHA, and ERP, the basic pH and EC were measured and recorded as 6.5 pH and 0.52 dSm^{-1} EC in compost, sand and coir dust mixture. The first dose of Azolla blended fertilizers for every treatment was applied into the potting mixture as a basal dressing right before transplanting, and subsequent applications were then applied as top dressings once every two weeks throughout the growing period.

Growth parameters, including plant height, number of leaves, branches, leaf length, leaf width, and stem diameter, were measured at the vegetative (1–35 days after transplanting; DAT), reproductive (36–70 DAT), and harvesting stages (71–112 DAT). Yield parameters, such as the number of days to first flowering, number of flowers, pods, pod length, pod girth, and fresh pod weight, were recorded to evaluate the impact of the treatments on chilli productivity (Ruhunuge *et al*., 2021).

Data analysis

Data were analyzed using one-way ANOVA in Minitab. Mean comparisons were conducted using Tukey's test and Dunnett's multiple range test at a 5% significance level.

RESULTS AND DISCUSSION

Nutrient status of organic fertilizer

The nutritional composition of the dried Azolla powder used in this study included 2.20% Nitrogen, 1.06% P_2O_5 and 1.96% K_2O. The organic carbon content was 4.12%, and the moisture content was 4.4%. A recent study found that the nitrogen percentage in Azolla range from 4 – 6 % (Marzouk *et al*., 2023), percentage of K_2O in rice husk ash burned at 600 ^{0}C was 2.57% (Mbakaan *et al*., 2014) and but values can vary from 0.06% to as high as 13% depending on factors like the rice variety, geographic location, and the temperature and duration of burning.

Plant height

The application of Azolla in powder form, blended with ERP and PHA, had a significant effect ($P < 0.05$) on plant height across the reproductive and harvesting stages in T2, T3, and T4 (Figure 1). At the reproductive (36 to 70 DAT) stage, the highest average plant height (14.30 cm) was recorded in T4 (9 Azolla:3 ERP:3 PHA), while the lowest (9.58 cm) was observed in the control treatment (T1). This trend continued through the reproductive and harvesting stages (71 to 112 DAT), with T4 consistently outperforming other treatments, reaching a maximum height of 88.25 cm at the harvesting stage. The increase in plant height observed in T4 can be attributed to the nitrogen supplied by Azolla, which promotes vegetative growth by enhancing cell division and elongation. (Setiawati *et al*., 2018). Similar findings were reported by (Jayamangkala *et al*.,2015)

observed that organic fertilizers such as compost and tea manure significantly enhanced plant height in broccoli by contributing essential nutrients. In addition, Raffaella *et al.* (2015) suggested that incorporating Azolla into the growing media influences olive plant growth, resulting in greater height.

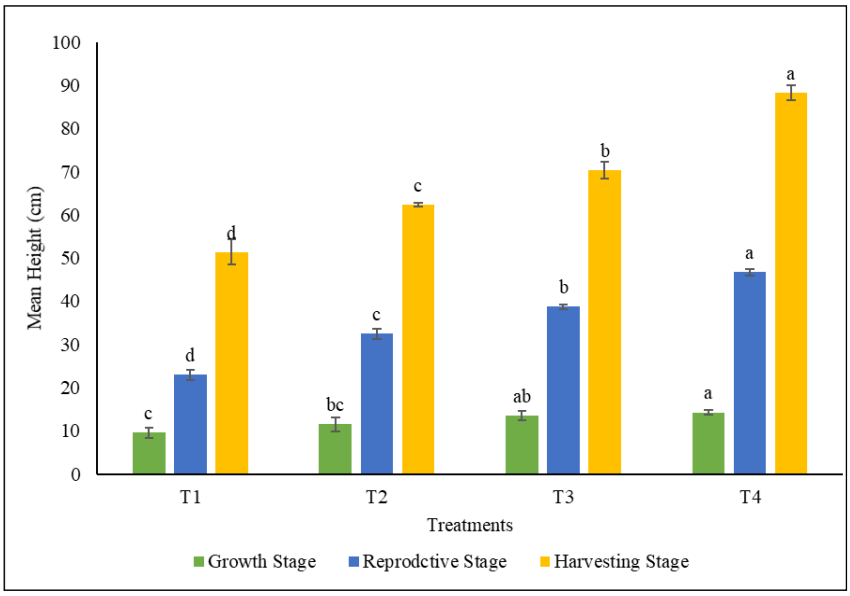

Figure 1: Effect of different fertilizer application rates on the plant height at vegetative, reproductive, and harvesting stages. Means that do not share the same letters are significantly different at the 0.05 level of probability according to Duncan's Multiple Range Test. All values represent the mean values of four replicates (mean ± SD)

Number of leaves

The number of leaves per plant showed significant ($P<0.05$) variation among treatments. Plants in T4 exhibited the highest number of leaves at all growth stages, with 9, 115, and 195 leaves observed at the growth, reproductive, and harvesting stages, respectively. In contrast, the lowest average number of leaves, 5, 30, and 78 leaves, were recorded in the T1 at the respective stages. This increase in leaf number may be attributed to the role of Azolla application in powder form, which enhances nitrogen availability in the soil and promotes its absorption by the roots, leading to improved vegetative growth. These results agreed with those of Riaz *et al.* (2022), who suggested that Azolla application provides essential nutrients, including nitrogen, necessary for plant growth. Additionally, Gopika *et al.* (2020) highlighted that that the Azolla application significantly increased the number of leaves in tomato plants due to its nutrient-rich composition. The significant increases in plant height and leaf number observed in T4 highlight the effectiveness of Azolla-enriched organic fertilizer in enhancing vegetative growth, likely due to its high nitrogen content and improved nutrient availability.

Number of branches

At the growth stage, there was a significant difference ($p<0.05$) in the average number of branches per plant between T4 and T1. The highest number of branches at the growth stage was recorded at T4 as 4, whereas the least number of branches was recorded at T1 as 0. There was no significant difference ($p>0.05$) between T2 and T3, but T4 showed a significant difference compared to T1, T2, and T3 at the reproductive stage. At the harvesting stage, the highest number of branches (82) was recorded in T4, while the least (32) was recorded in T1, with a significant difference ($p<0.05$) between T1 and T4. The substantial increase in the number of branches, particularly in T4, could be attributed to the application of powdered Azolla, which enhances nutrient availability and promotes

vegetative development. This observation aligns with the findings of Hanafy *et al*. (2018), who reported that the number of branches is significantly influenced by the foliar spray application of Azolla bio-fertilizer to tomato plants.

Leaf length and width

Leaf dimensions, including length and width, were significantly influenced ($P < 0.05$) by the application of Azolla-based fertilizer. The highest average leaf length (15.075 cm) and width (6.00 cm) were recorded in T4, while the lowest values were observed in T1 (12.5 cm and 4.85 cm, respectively) at the harvesting stage. This improvement in leaf size can be attributed to the increased availability of nitrogen and other essential nutrients provided by the Azolla-based treatment, which supports cell expansion and leaf development. These findings align with those of Rajeevan *et al*. (2024), who reported that chilli plants treated with Azolla solution combined with vermicomposting exhibited significantly larger leaf dimensions. Maswada *et al*. (2021) reported that Azolla, as a biofertilizer, can significantly improve maize growth performance under nitrogen-deficient conditions.

Stem diameter

Stem diameter, an indicator of plant strength and nutrient uptake, was significantly higher ($P < 0.05$) in T4 (7.535 mm) compared to the control (5.617 mm) at the harvesting stage (Figure 2). Across all stages, T4 consistently shows the highest mean stem diameter, suggesting it may be the most effective fertilizer treatment. The nitrogen-rich Azolla, combined with the phosphorus from ERP and potassium from PHA, supported robust stem development and nutrient transport within the plant. This increase in stem diameter can be attributed to improved nutrient availability and soil microbial activity facilitated by the organic fertilizer. Similar results were reported by Jama *et al*. (2023), who found that Azolla-based fertilizers significantly increased stem diameter in vegetable crops. (Lestari *et al*., 2020) has shown that Azolla, combined with reduced urea doses, significantly enhances lettuce growth and yield parameters, including stem diameter, demonstrating the potential of organic fertilizer to reduce reliance on synthetic nitrogen fertilizer.

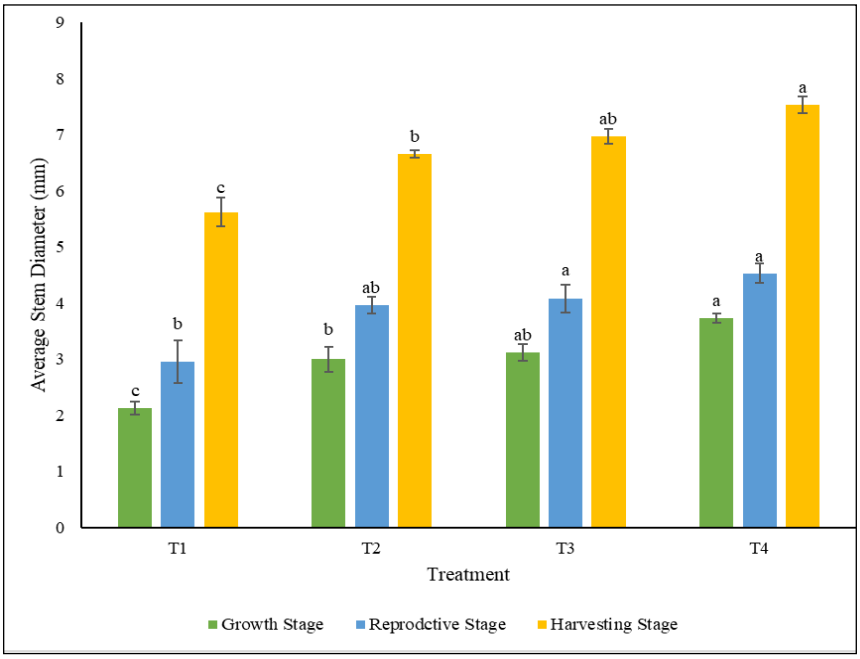

Figure 2: Effect of different treatments on the stem diameter at growth, reproductive and harvesting stages. Means that do not share the same letters are significantly different at the 0.05 level of probability according to Duncan's Multiple Range Test. All values represent the mean values of four replicates (mean ± SD).

Number of days to first flowering

The number of days to first flowering was significantly reduced ($P < 0.05$) in T4 (60 days) compared to the control (76.5 days). T2 (64.5 days) and T3 (61.5 days) showed a moderate reduction in flowering time compared to the control (Table 1**)**. This early flowering in T4 was enhanced by the essential nutrients supplied through the fertilizer mixture, particularly nitrogen from Azolla, phosphorus from rock phosphate, and potassium from rice husk ash, which promote faster vegetative growth and an early transition to the reproductive stage. These results were consistent with the findings of Kumar *et al*. (2016), who reported that combinations of organic manures enhance nutrient availability and promote faster vegetative growth and earlier flowering. These results are consistent with the findings of Arfiani *et al*. (2018), who reported that Azolla compost significantly reduced the time to flower formation in broccoli, possibly because it provides nutrients slowly to the plant, resulting in earlier flowering. Early flowering is a desirable trait in chilli cultivation as it may lead to earlier fruiting, extended harvesting periods, and potentially higher yields.

Table 1: Effect of different treatments on number of days to first flowering, number of flowers and mean number of pods per plant in *MICHHY0I* green chilli

Treat-ments	Number of Days to first flowering	Mean number of flowers/ plants	Mean number of pods/plants
T_1 (Control)	76.50 ± 0.86^{a}	34.75 ± 3.57^{a}	7.50 ± 1.55^{a}
T_2	64.50 ± 0.86^{b}	53.50 ± 3.93^{b}	20.0 ± 2.38^{b}
T_3	61.50 ± 1.50^{bc}	62.25 ± 3.75^{bc}	30.0 ± 1.83^{b}
T_4	60.00 ± 0.00^{c}	84.25 ± 6.30^{c}	45.75 ± 3.52^{c}

Means that those that do not share the same letters are significantly different

Number of flowers per plant

The number of flowers per plant was significantly higher ($P < 0.05$) in T4 (84.25 flowers) than in the control (34.75 flowers; Table 1**)**. This increase in flower production can be attributed to the nitrogen-rich Azolla, which contains ERP and PHA, thereby supporting flower initiation and development. These results were consistent with those of Forghan and Mahboub (2018), which showed that the number of flowers increases with increasing doses of Azolla compost. Similar findings were reported by Lubis *et al*. (2021), who observed that the application of Azolla significantly increased the number of flowers in rice plants due to improved nutrient availability.

Number of pods per plant

The number of pods per plant was significantly higher ($P < 0.05$) in T4 (45.75 pods) compared to the control (7.50 pods). Treatments T3 and T2 also have a higher number of pods per plant than the control (Table 1**)**.

However, their performance remained considerably lower than T4. This increase in pod production can be linked to the balanced nutrient supply from Azolla, ERP, and PHA, which supports fruit set and development. These findings were consistent with previous studies by Lubis *et al*. (2021), who reported an increase in rice tillers following the application of urea, cow manure, and Azolla. Similarly, Hanafy *et al*. (2018) observed positive effects of organic nutrient sources on reproductive growth. Furthermore, Intana *et al*., (2023) found that Azolla-based fertilizers significantly increased pod production in melon plants.

Single pod weight

While there was no significant difference ($P > 0.05$) in single pod weight among the treatments, T4 recorded the highest pod weight (5.34 g), followed by T3 (5.04 g), T2 (4.59 g), and T1 (4.59 g) (Table 2**)**. The slight increase in pod weight in T4 can be attributed to the improved nutrient availability, particularly nitrogen and potassium, which support pod development. These findings are consistent with those of Widiastuti *et al*. (2017), who reported that the application of Azolla improved fruit weight in spinach and radish.

Pod length

Pod length was significantly influenced ($P < 0.05$) by the Azolla-based fertilizer, with T4 recording the highest pod length (12.37 cm), followed by T3 (12.00 cm), T2 (11.57 cm), and T1 (9.30 cm) (Table 2). This increase in pod length can be attributed to the balanced release of essential macro- and micronutrients into the soil by Azolla, ERP, and PHA, which supports cell elongation and pod development. Similar results were reported by Jama *et al.* (2023), who found that Azolla-based fertilizers significantly improved fruit length in vegetable crops, such as radish tubers and spinach, in peat soil.

Table 02: Effect of different treatments on pod length, pod girth, single pod weight, yield per plant and total yield in *MICHHY0I* green chilli

Treatment	Pod length (cm)	Pod girth (mm)	Single pod weight (g)	Total yield (t/ha)*
T1	9.300±0.0913[a]	8.168±0.113[a]	4.588±0.295[a]	3.411±0.297[a]
T2	11.575±0.466[a]	9.080±0.189[b]	4.598±0.110[a]	12.357±0.539[b]
T3	12.000±0.091[a]	9.335 ±0.101[b]	5.040±0.119[a]	16.860±1.180[b]
T4	12.375±0.111[b]	10.360±0.150[c]	5.343±0.220[a]	29.840±2.480[c]

*Total yield was calculated based on yield per plant. Means that those that do not share the same letters are significantly different

Pod girth

Pod girth was significantly higher ($P < 0.05$) in T4 (10.36 mm) compared to the control (8.16 mm) (Table 2). Higher significant pod girth in treatment four, possibly due to the application of Azolla-based organic fertilizer that releases nutrients that were readily available to the plant in the right amount. These findings were in line with Intana *et al.* (2023), who found that fermented and fresh Azolla served as a plant growing medium, resulting in greater fruit perimeter and fruit weight. These findings are also consistent with those of Rajeevan *et al.* (2024), who reported that the application of Azolla significantly increased pod girth in chilli plants.

Yield per plant

The highest yield per plant (108.69 g) was recorded in T4, while the lowest (13.81 g) was observed in the control (Figure 3). This significant increase in yield under T4 can be attributed to the combined effect of Azolla, ERP, and PHA, which together provided a balanced and sustained nutrient supply, leading to enhanced soil fertility and improved plant performance (Shaji *et al.*, 2021). The improved availability of essential nutrients such as nitrogen, phosphorus, and potassium likely supported vigorous vegetative growth, efficient flowering, and better pod development, culminating in higher yields. These results align with the findings of Seleiman *et al.* (2022), who reported that the application of Azolla-based fertilizers significantly enhanced rice yields.

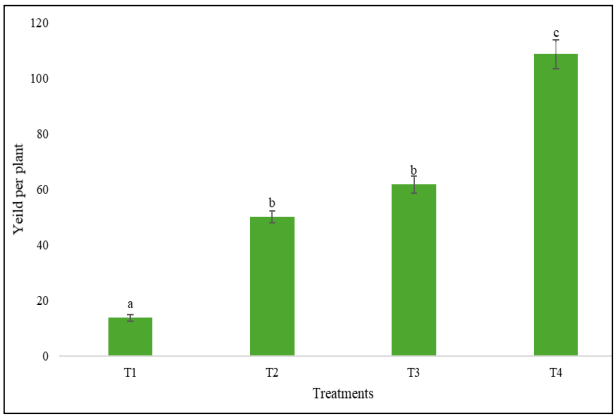

Figure 3: Effect of different fertilizer application rates on the plant yield. Means that do not share the common letters are significantly different at the 0.05 level of probability according to Duncan's Multiple Range Test. All values represent the mean values of three replicates (mean ± SD)

Total yield

The highest yield per plant (108.69 g) and total yield (29.84 t/ha) were recorded in T4, whereas the lowest values were observed in the control treatment, with only 13.81 g per plant and 3.411 t/ha total yield (Table 2). This significant increase in yield can be attributed to the combined effect of Azolla, ERP, and PHA, which together provided a balanced and

sustained nutrient supply, thereby improving soil fertility and overall plant performance. The nitrogen-fixing ability of Azolla, along with the slow-release phosphorus from ERP and the potassium content of PHA, created an optimal environment for nutrient uptake, vegetative growth, and fruit development in chilli plants. These findings are consistent with those of (Jama *et al*., 2023) who reported that Azolla-based fertilizers significantly increased crop yield in vegetable production because of Azolla mineralized N much more rapidly than compost. Findings by Intana *et al*. (2023) recommend using fresh Azolla in a soilless culture system to improve melon production, which is in line with this research finding. According to the recommendation of the Department of Agriculture, Sri Lanka, the F1 hybrid variety *MICHHY01* has a maximum potential yield of 32.00 t/ha when grown under optimized conventional chemical fertilizer management.

CONCLUSION

The results of this study clearly demonstrate that the application of Azolla-based organic fertilizer, particularly at a ratio of 9 Azolla: 3 ERP: 3 PHA (T4), significantly enhanced the growth and yield performance of chilli plants. The improved nutrient availability, particularly nitrogen, phosphorus, and potassium, contributed to vigorous vegetative growth, earlier flowering, and substantially higher yield. However, because this study was limited to a single pot experiment and did not include a field-level trial or an analysis of soil health, this fertilizer mixture cannot yet be formally recommended for commercial use. Further field studies and detailed soil tests over multiple seasons are necessary to confirm these results before making agricultural recommendations.

AUTHOR CONTRIBUTION

BMHSB, KMWR, and KHTK conceptualized and designed the study. BMHSB conducted the research and analyzed the data. BMHSB drafted the manuscript, and KMWR and KHTK critically reviewed and revised the manuscript.

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