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Role of Leguminosae Root Fermentation on Vegetative Growth of Cayenne Pepper (*Capsicum frutescens* L.) After Flooding Stress

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Abstract

Flooded cayenne pepper (Capsicum frutescens L.) experiences oxidative stress causing growth disturbances, delayed and reduced yields, and even death. The success of cayenne pepper in the recovery process determines the overall success of cayenne cultivation after flooding stress. Leguminosae roots are associated with rhizosphere bacteria which are beneficial for the recovery process of cayenne pepper after flooding stress. The purpose of this study was to determine the effect of leguminosae root fermentation on the vegetative growth response of cayenne pepper after flooding stress. This research used a Completely Randomized Design (CRD) with 5 treatments, namely T1 cayenne pepper without flooding and biofertilizer, T2 cayenne peppers without flooding and biofertilizer applied, T3 cayenne peppers flooded without biofertilizer application, T4 cayenne peppers flooded and 50 % biofertilizer applied, T5 cayenne peppers flooded and 100 % biofertilizer applied. Partial flooding was carried out for 48 hours and biofertilizer application was carried out after 24 hours of drainage. The results showed that the application of biofertilizer had a significant effect on the vegetative growth of cayenne pepper after flooding stress on the parameter of average number of leaves when applying 100 % biofertilizer. This research concludes that Leguminosae root fermentation plays a role in the vegetative growth of cayenne pepper after flooding stress, so it has the potential to be used as a biofertilizer

Keywords: *Biofertilizer; Cayenne pepper; Flooding; Leguminosae*

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INTRODUCTION

Climate change has an impact on rainfall anomalies that cause floods or droughts. This has an impact on the physical, chemical, and biological conditions of agricultural land (Pais et al., 2023; Xu et al., 2023; Yu et al., 2022). Flooding and poor drainage cause flooding on agricultural land (Zhang et al., 2021). Flooding at a certain level and duration causes changes in soil microbial status and soil physico-chemistry (Pais et al., 2023; Xu et al., 2023; Yu et al., 2022). Soil microbes have a very important role in agricultural land, including as chelating agents and degrading toxic compounds, providing nutrients needed by plants, protecting roots from pests and diseases, and providing phytohormones to stimulate plant growth (Toor & Adnan, 2020). Flooding affects the presence and activity of soil bacteria (Yu et al., 2022). The uptake of nutrients C and N has decreased, while toxic compounds and the activity of anaerobic bacteria have increased due to flooding on agricultural land (Zhang et al., 2021).

Plants respond negatively to flooding stress on agricultural land (Xu et al., 2023). This response varies at the species, cultivar, and even individual levels (Pahlevi et al., 2019). Plant responses to flooding stress begin with hypoxia and anoxia conditions in plant roots that cause the production of ACC (1-aminocyclopropane-1-carboxylic acid) which is mobilized to plant shoots for ethylene biosynthesis (Pudjiwati & Pongliku, 2021). In addition, flooding stress causes aerobic respiration resulting in an energy crisis in plant roots. So to obtain energy, it is obtained through changing aerobic respiration to anaerobic which will accumulate Reactive Oxygen Species (ROS) causing cayenne pepper to experience oxidative stress (Pahlevi et al., 2021). This oxidative stress will disrupt plant growth and development both vegetatively and generatively (Pahlevi & Kurniahu, 2022; Raras et al., 2021). Even extreme flooding combined with traits that are intolerant of flooding stress will cause death in cultivated plants (Pahlevi et al., 2019).

Cayenne pepper (*Capsicum frutescens* L.) is an important horticultural commodity in Indonesia. The success of the cayenne pepper harvest is one of the factors supporting the economic stability of the people in Indonesia (Wisnujati & Siswati, 2021). Cayenne pepper (*C. frutescens* L.) that experiences flooding stress will show growth disturbances, harvest delays, and death. Various cayenne pepper cultivars have different responses to flooding stress. Several studies on the response of cayenne pepper to flooding stress have been reported including epinasty response (Pahlevi et al., 2018), inhibition of plant height, decreased number of fruits (harvest), and number of branches (Pahlevi et al., 2021), wilting to death of cayenne pepper plants (Pahlevi et al., 2019). These disorders can be economically detrimental to farmers.

Farmer losses can be reduced if cayenne pepper affected by flooding quickly recovers to avoid crop failure or death. Cayenne pepper naturally can recover after flooding stress (Raras et al., 2021). However, this process takes a longer time, increasing the duration of cayenne pepper cultivation. Therefore, efforts are needed to accelerate the recovery process of cayenne pepper plants after flooding stress. A study related to the recovery process of cayenne pepper has been carried out (Raras et al., 2021). In addition, studies overcome flooding stress in plants have also been carried out including the application of silica fertilizer in red chili (*C. annuum* L.)

(Budinuljanto et al., 2022) and exogenous calcium ($CaNO_3$) in tobacco plants (*Nicotiana tabacum* L.) (Hidayat et al., 2023). However, the use of inorganic fertilizers has a negative impact on agricultural ecosystems. Therefore, this study was conducted to determine the role of biofertilizer from Leguminosae root fermentation for the vegetative growth of cayenne pepper under flooding stress. Biofertilizer is a fertilizer that contains microbes that play a role in helping increase plant growth. So the use of biofertilizer in post-flooding cayenne pepper will accelerate the recovery process from oxidative stress and improve soil conditions to support sustainable agriculture.

METHOD

This research is an experimental study that uses a Completely Randomized Design (CRD) to determine the effect of applying biofertilizer roots from Leguminosae on the vegetative growth of cayenne pepper plants (*Capsicum frutescens* L.) after flooding stress. This study consisted of five treatments with five replicates of each treatment , namely T1 cayenne peppers without flooding and biofertilizer, T2 cayenne peppers without flooding and biofertilizer applied, T3 cayenne peppers flooded without biofertilizer application, T4 cayenne peppers flooded and 50 % biofertilizer applied, T5 cayenne peppers flooded and 100 % biofertilizer applied. The research steps taken are as follows,

Biofertilizer Preparation

The roots of Leguminosae plants (Fabaceae family) used were Orok-orok (*Crotalaria juncea*), Peanut (*Arachis hypogaea* L.), and Lamtoro (*Leucaena leucocephala*). The roots and soil around the roots of these plants were weighed and added with molasses and rice bran as well as phosphate buffer pH 7. Then fermented for 7 days at room temperature (27 – 30 ºC) (Andriani et al., 2021; Kurniahu, 2023; Kurniahu et al., 2022).

Planting and Acclimatization of Cayenne Pepper (*Capsicum frutescens* L.) Pre-Flooded

Ori cultivar cayenne pepper seedlings were planted in a mixture of soil, manure, compost, and husk charcoal in a ratio of 3:1:1:1 modified from Pahlevi et al., (2021). Furthermore, acclimatization was carried out for 45 days in the greenhouse. Cayenne pepper plants were watered every two days with 450 ml of tap water. Weed and pest control was done mechanically.

Flooding treatment on cayenne pepper (*Capsicum frutescens* L.)

Flooding treatment on cayenne pepper (*Capsicum frutescens* L.) was done 45 Days After Planting (DAP). Flooding treatment on cayenne pepper was done partially as high as about 15 cm from the surface of the planting media. Cayenne pepper was flooded for 48 hours and then drained for 24 hours. Then biofertilizer from

Leguminosae roots was applied to the cayenne pepper after experiencing flooding stress.

Data collection

The vegetative growth response of cayenne pepper was observed after 7 days post-treatment and repeated weekly for 5 weeks. Vegetative growth parameters observed were plant height, number of leaves, and number of branches. Plant height was measured using a ruler starting from the top surface of the planting medium to the growing point, while the number of leaves and the number of branches were counted using a hand tally counter.

Data analysis

The data obtained in the form of plant height, number of leaves, and number of branches every 7 days from 45 until 80 DAP. Statistical analysis using the Manova test on SPSS 26 software with a significance value of 0.05 to determine the effect of biofertilizer from fermented Leguminosae roots on the vegetative growth of cayenne pepper after flooding stress. Furthermore, if the analisys results show a significant effect, it will be continued with the LSD (Least Significant Difference) test to determine the most optimal treatment effect on the vegetative growth of cayenne pepper after flooding stress.

RESULT AND DISCUSSION

Cayenne Pepper (*Capsicum frutescens* L.) Height

The average height of cayenne pepper is presented in **Table 1** and **Figure 1**. Plant height was measured using centimeters. This study consisted of two pre-treatment groups, namely without flooding (T1 and T2) and with flooding (T3, T4, and T5) and 5 post-treatment groups. Plant height was measured 7 days after the treatment of fermented Leguminosae root fertilizer which was then repeated every 7 days for 5 times until 35 days after treatment. The average results of the measurement of cayenne pepper height after treatment are as follows:

Table 1. The average cayenne pepper height after treatments (cm)

Treatments	Days After Treatment				
	7	14	21	28	35
T1	43.0^{ac}	45.0^{ab}	50.4^{a}	52.8^{ac}	57.2^{a}
T2	46.6^{c}	51.4^{a}	55.8^{a}	57.4^{c}	61.2^{a}
T3	35.8^{ab}	37.0^{b}	37.8^{b}	39.6^{b}	40.8^{b}
T4	32.1^{b}	35.4^{b}	38.2^{b}	41.8^{ab}	44.0^{b}
T5	35.4^{ab}	38.2^{b}	38.6^{b}	39.8^{b}	42.4^{b}

Notes: Different letters (a,b,c) in the same column indicate significant differences in the LSD test.

The results of the Manova statistical test ($P < 0.05$) showed that the treatment of biofertilizer of fermented Leguminosae plant roots showed a significant effect on the average height of cayenne pepper after flooding stress every 7 days. Based on the

LSD test (P>0.05), it is known that the T2 treatment (plants without flooding and 100 % biofertilizer application) shows the most optimal response (7, 14, 21, 28, and 35 days after treatment). The LSD test results also showed that cayenne peppers that were not flooded either applied within biofertilizer or without biofertilizer (T1 and T2) did not give a significant difference. However, the two treatments were different from the cayenne pepper group that was flooded (T3, T4, and T5). While the results of the LSD test on the group of plants that were flooded both those that were not applied with biofertilizer and those that were applied with various concentrations of biofertilizer (T3, T4, and T5) did not show significant differences. This shows that flooding affects the height of the cayenne pepper. Pahlevi et al., (2021) stated that periodic flooding of three cayenne pepper cultivars *Sret*, *Mhanu,* and *Cakra Hijau* caused growth inhibition, one of which was plant height.

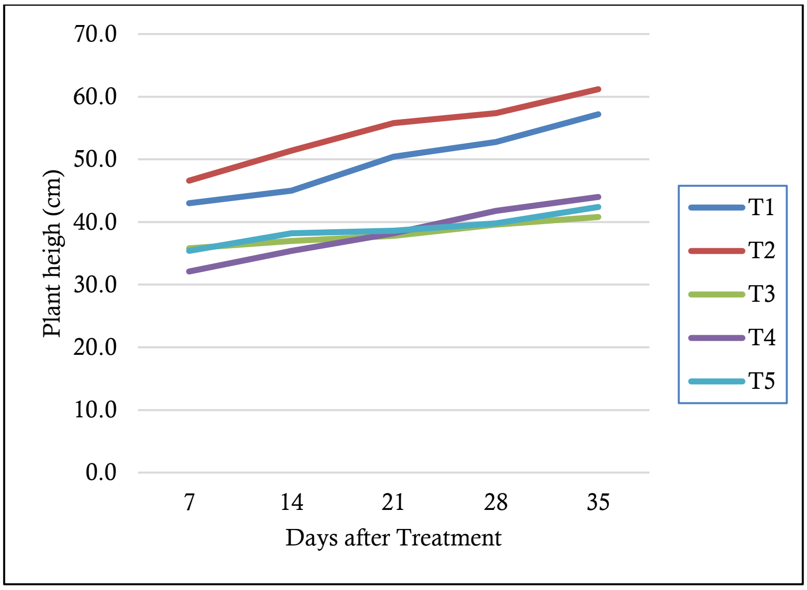

Figure 1. Height diagram of cayenne pepper after treatment

Based on **Table 1** and **Figure 1**, it is known that fertilizer application increases the response of cayenne pepper height after flooding stress. In the observation 7 days after the treatment, the lowest plant height was T4 (flooding and 50 % fertilizer application). While, cayenne pepper that is not flooded and applied fertilizer has a high average above the T4 and T5 treatments (flooded and applied fertilizer). This is because, at the time of treatment, the cayenne pepper was already 45 DAP, whereas the T4 and T5 treatments had a lower average than the T3 average. However, at the end of observation or 35 days after treatment, it was known that the average height of cayenne pepper in T3 was lower than in T4 and T5 even though based on the LSD test the three treatments did not show significant differences. This shows that the application of biofertilizer gives a positive response to the height of cayenne pepper and the rate of height increases in cayenne pepper after flooding stress with the application of biofertilizer. This is according to a previous study that states that the addition of biofertilizer from plant roots (PGPR) can increase the height gain of cayenne pepper and red pepper plants under normal conditions (Ollo et al., 2019).

The mechanism of PGPR (Plant Growth Promoting Rhizobacteria) in spurring plant height increases is because PGPR can produce auxin hormones in the plant root environment (Kurniahu et al., 2018). The auxin has a role in triggering cell division and elongation of meristem cells to accelerate the addition of plant height (Siregar et al., 2024). On the other hand, Insani et al., (2021); Raras et al., (2021); and Sayekti et al., (2022) stated that the recovery process of plants after flooding stress takes time so that the morpho-physiological response of plants will experience significant differences at the end of the recovery period.

Table 2. The average number of leaves of cayenne pepper after treatment

Treatments	**Days After Treatment**				
	7	**14**	**21**	**28**	**35**
T1	14.0^{a}	15.0^{ac}	19.0 ^{a}	19.2^{a}	19.6^{a}
T2	15.0^{a}	17.6 ^{c}	21.2 ^{a}	20.2^{a}	21.0^{a}
T3	9.6^{b}	9.0 ^{b}	8.4^{bc}	8.4^{b}	9.0^{b}
T4	9.6^{b}	12.4^{ab}	14.8^{ac}	16.0^{a}	16.2^{b}
T5	10.4^{b}	12.2^{ab}	12.8^{ac}	18.0^{a}	16.6^{b}

Notes: Different letters (a,b,c) in the same column indicate significant differences in the LSD test.

Manova statistical test (P<0.05) on the observation parameter of the average number of leaves showed that the application of biofertilizer from Leguminosae root fermentation had a significant effect on the average number of leaves of cayenne pepper after flooding stress. **Table 2** shows the results of the LSD test (P>0.05) indicating that cayenne pepper in treatment T2 (without flooding and applied 100 % biofertilizer) gave the response of the average number of leaves the most while the average number of leaves the least was in treatment T3 (flooding without biofertilizer application). The application of biofertilizer concentrations of 50 % (T4) and 100 % (T5) on cayenne pepper after flooding stress has a mean number of leaves that is not significantly different from the treatment without flooding either given biofertilizer (T2) or not (T1). Meanwhile, cayenne pepper that was flooded but not applied to biofertilizer (T3) had an average number of leaves that was significantly different from the other treatments (T1, T2, T4, and T5) at observations 7, 14, 28, and 35 days after treatment. Whereas on day 21 T3, T4, and T5 had an average number of leaves that were not significantly different.

This result shows that iofertilizer from fermented Leguminosae roots can accelerate the recovery process of cayenne pepper (*C. frutescens* L.) since the first week after treatment. According to Ollo et al., (2019), the application of biofertilizer from plant roots (PGPR) containing beneficial bacteria will increase the average number of red chili leaves compared to the control under normal conditions. Giving PGPR with the right dose can make optimal leaf number growth because it can produce and change the concentration of phytohormones and mobilize and facilitate the absorption of nutrients needed in plant growth including an increase in the number of leaves (Andriani et al., 2021; Kurniahu et al., 2018). Bacteria in the root system of Leguminosae plants also can optimize the absorption and utilization of N nutrients needed in plant vegetative growth including leaf growth and chlorophyll formation (Koryati et al., 2022).

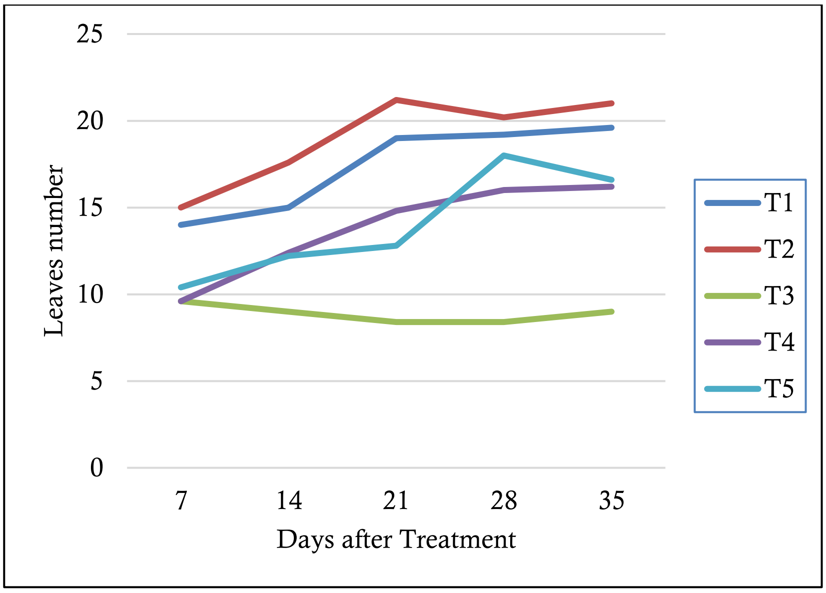

Figure 2. Leaves number diagram of cayenne pepper after treatment

The graphs in **Figure 2** and **Table 2** show that there are fluctuations in the average number of leaves. This is because, in addition to experiencing leaf growth, it also experiences shedding. Under normal conditions, old leaves will fall and be replaced with young leaves (Kusdiana, 2020). However, under flooding stress conditions, plants experience an increase in ethylene gas in the shoots due to the mobilization of ACC formed in the roots when experiencing oxygen deprivation due to flooding (Pudjiwati & Pongliku, 2021). This causes the leaves to wilt and then fall before aging. This condition is exacerbated by the inhibition of nutrient absorption so that the formation of new leaves is inhibited. According to Zhang et al., (2021) waterlogging of cotton roots inhibits nutrient uptake, and disrupts C and N metabolism, which in turn disrupts sugar and protein content. Therefore, the accumulated number of leaves decreased under flooding stress conditions.

Table 3. The average number of branches of cayenne pepper after treatment

Treatments	**Days After Treatment**				
	7	**14**	**21**	**28**	**35**
T1	0.0[a]	0.4[a]	3.2[a]	4.2[a]	4.0[a]
T2	0.0[a]	0.4[a]	3.8[a]	5.0[a]	7.6[a]
T3	0.8[a]	0.8[a]	1.0[a]	1.0[a]	1.0[a]
T4	0.0[a]	0.6[a]	2.0[a]	3.4[a]	4.2[a]
T5	0.4[a]	0.8[a]	2.0[a]	3.8[a]	4.2[a]

Notes: Same letter (a) in the same column indicates non-significant difference in LSD test.

The results of the Manova statistical test ($P>0.05$) on the parameter of the number of branches showed that the application of biofertilizer from fermented

Leguminosae plant roots does not have a significant effect on the number of branches in cayenne pepper after flooding stress. This is different from the study by Guntoro & Bahri (2023) the application of biofertilizer (PGPR) can increase the number of branches of red chili under normal conditions. According to Pahlevi et al., (2021), periodic flooding has a significant effect on the branch of the cayenne pepper cultivar *Sret* compared to the control. The difference in results is due to the different types of flooding stress, different cultivars, and different data collection times. This study used *ORI* cultivar and the flooding stress was partial flooding for 48 hours. Pahlevi et al., (2019) stated that different plant cultivars have different responses in the face of flooding stress, different stresses will respond differently to the same cultivar.

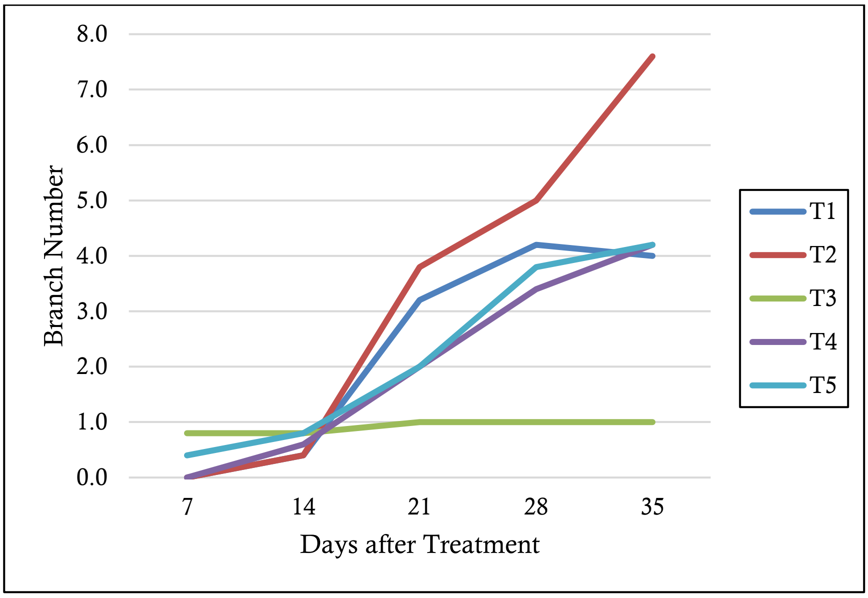

Figure 3. Branch number diagram of cayenne pepper after treatment

The application of biofertilizer-fermented Leguminosae roots can increase the average number of branches even though based on the results of statistical tests it has no significant effect (**Table 3**). This is because the formation of branches requires a longer process so the observation for 35 days after treatment has not shown a significant difference. However, **Figure 3** shows that treatment T2 (without flooding and 100 % biofertilizer application) had the highest average number of branches while the least was treatment T3 (flooding without biofertilizer application). Meanwhile, treatments T4 and T5 (flooding and applied biofertilizer 50 % and 100 %) had the same average number of branches and more than treatment T1 (without flooding and biofertilizer). According to Insani et al., (2021); Raras et al., (2021); Sayekti et al., (2022) plants naturally can recover after flooding stress, but this process takes time so not all morphological responses are observed at the beginning of the recovery process.

Biofertilizer is a fertilizer that contains microorganisms that function to provide nutrients for plants, restore the natural nutrient cycle of the soil, and maintain soil health (Benu et al., 2023). Biofertilizers made from fermented plant roots contain rhizosphere bacteria that can mobilize and provide nutrients to plants, increase the concentration of phytohormones, and have antagonistic properties against pathogenic bacteria in plants (Nurhadi & Noor, 2022). Leguminosae roots contain N-fixing bacteria that are well-used as biofertilizers (Koryati et al., 2022). Therefore, this study utilizes the roots of Leguminosae plants as raw material for biofertilizer in cayenne pepper after flooding stress.

Flooding stress in cayenne pepper causes cayenne root metabolism disrupted due to an energy crisis caused by a lack of oxygen. This oxidative stress will disrupt the growth of cayenne pepper, especially in the vegetative phase (Pahlevi et al., 2018; 2021; 2019; Pahlevi & Kurniahu, 2022). Naturally, cayenne pepper can carry out the recovery process (Insani et al., 2021; Raras et al., 2021). The success of the recovery process, especially in the vegetative phase, determines the survival and yield of cayenne pepper. Soil microbial groups in the rhizosphere area can increase plant resistance to abiotic stress (Asra et al., 2024). The bacteria can produce antioxidants to detoxify ROS which improves osmotic adjustment in plants and also increases IAA (Indole Acetic Acid) which spurs root growth, produces root surface area, root length, and the number of root tips, and increases nutrient absorption in roots (Koza et al., 2022). Rhizosphere bacteria are also able to produce the enzyme ACC (1-Aminocyclopropane-1-carboxylic acid) deaminase which can inhibit the formation of ethylene (Singh *et al.*, 2022) With the inhibition of ethylene formation, the morpho-physiological response of the plant in the form of epinasty, wilting and leaf fall can be suppressed.

Other studies also mention that rhizosphere microbes can increase the growth and development of root hair because this microbial group can increase phytohormones so that the roots can play a good role in absorbing nutrients (Hariyadi et al., 2021). The significant response in the form of the average number of leaves shows that the application of fermented Leguminosae roots can make cayenne pepper plants increase the concentration of phytohormones and better absorption of nutrients after flooding stress. According to Kurniahu (2023) soaking cayenne pepper seeds in a solution containing rhizosphere bacteria (PGPR) can increase the number of germinations and the quality of cayenne pepper seedlings. The addition of PGPR can maximize the growth of cayenne pepper both vegetative and generative growth when compared to without the addition of PGPR (Murti et al., 2024). Rhizosphere bacteria can improve root access to nutrients and water and increase nutrient translocation to above-ground parts of the plant, leading to an overall increase in plant growth and yield (Khan *et al.*, 2021).

CONCLUSION

Based on the results of the study, it can be concluded that biofertilizers from fermented Leguminosae roots have the potential to be used as agents to accelerate the recovery process of cayenne pepper after flooding because the results of this biofertilizer study was able to increase the vegetative growth of cayenne pepper after flooding stress, especially in the parameter of the average number of leaves which increased significantly.

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