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The Effect of Various Microbiomes as Soil Probiotics to the Thinning Corn Plants [*Zea mays* (L.) Saccharata] Forage on Growth, Production and Forage Quality

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ABSTRACT

Background: This study aims to evaluate the use of microbiomes as probiotics in corn plants (*Zea mays* L. Saccharata) resulting from thinning forage (35 days after planted). The use of microbiomes as plant probiotics is expected to provide an alternative environmentall friendly solution in increasing the productivity of thinning results of corn plants and agricultural sustainability. This research was conducted at the Ciparanje research field and the animal feed crops laboratory, faculty of animal husbandry, padjadjaran university in Indonesia from August 2023 to February 2024.

Methods: The method used was experimental with a completely randomized design (CRD) with 3 microbiome probiotic treatments, namely J1 (Lactobacillus and Saccharomyces), J2 (mycofer probiotics (glomus etunicatum, glomus manihotis, gigaspora margarita, acaulospora sp) and J3 (combination of lactobacillus + saccharomyces + Bacterial Acidic Lactate (BAL) + mycofer). Each treatment was repeated 6 times. The research data were analyzed using the anova test and duncan's further test.

Result: The results showed that the treatment of various microbiomes gave significant effect on the plants height and the quality of corn forage (crude protein, phosphorus and calcium). The fresh weight and the dry weight production of forage gave the similar results.

Key words: Corn crop, Forage quality, Harvest result, Microbiome.

INTRODUCTION

Corn development offers excellent prospects as a cereal crop. Corn is a global crop and is cultivated by farmers for its maize yield (Indriani *et al.*, 2021). In supporting the increasing food needs, agricultural land per unit area is needed to achieve maximum efficiency and the highest quality products. Plant nutrition is one of the most important factors in controlling agricultural productivity and quality. The nutrient content in the soil affects the quality of the harvest. On permanent agricultural land, the soil will be very poor in nutrients and as a result, the poor quality and production. Therefore, soil fertilization, pest control, irrigation and agricultural activities make the soil more efficient. Fertilization among these activities is always a priority (Jote, 2023).

Planting sweet corn with one seed per hole produces higher productivity than planting two seeds per hole. Growth is greatly influenced by the number of seeds planted because it is related to the competition for nutrient absorption in the soil (Regyta *et al.*, 2023). In addition to the space where the plants grow, nutrient absorption is disrupted, there is also competition for sunlight for photosynthesis. According to Stansluos *et al.* (2024), the plant density for the sweet corn varieties used has a tolerance to obtain maximum results. Furthermore, according to Bisht *et al.* (2013) that high plant density results in a decrease in the content of macronutrients in sources and absorption during fertilizer application, either inorganically or variable. According to Shah *et al.* (2021) densely planted plants have a high risk of stem collapse and seed miscarriage.

Generally, planting sweet corn seeds in one hole contains two corn seeds which are left to become one plant in a few weeks. The removal of plants is seen to what extent the main plant can grow properly and requires uniformity in the growth of the main plant (which is not removed). The results of the removed plants are called thinning plants which are very good for feed because the age of the plants is very young with high crude protein and is preferred by livestock. According to Mukhtar *et al.* (2023) that corn leaves, in addition to their high crude protein, also have high palatability so that corn leaves are very good

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to give to livestock, especially young corn plants that are harvested.

Excessive or continuous inorganic fertilization will cause serious environmental problems such as accumulation of heavy metals, accumulation of nitrates (gases containing nitrogen and sulfur), eutrophication of water, toxicity to various beneficial microorganisms, loss of biodiversity and can cause global warming (Jote, 2023). Continuous use of artificial fertilizers in crop cultivation can raise concerns regarding soil degradation, loss of productivity and potential contamination of food with hazardous residues (Lalkhumliana *et al.*, 2025). Another disadvantage when inorganic fertilizers are used excessively, unabsorbed residues can damage groundwater through irrigation and rain. The acid content of inorganic fertilizers, such as hydrochloric acid and sulfuric acid, causes high soil acidity levels which can damage the nitrogen-fixing microbiome. This microbiome plays a major role in providing nitrogen needed by growing plants. According to Titirmare *et al*. (2023) that inorganic fertilizers contain all the essential nutrients that can be accessed earlier by plants. Continuous use of inorganic fertilizers alone can cause degradation of soil organic matter, soil acidity or alkalization, soil damage and environmental pollution, so an integrated or mixed inorganic and organic nutrient management system is an alternative system for sustainable and cost-effective soil management and the result is improvement of soil properties and increased soil fertility without affecting the environment. Furthermore, according to Manzoor *et al.* (2024) that integrated/combined fertilization of both inorganic and organic fertilizers, can show better performance in improving soil properties and plant growth and quality. In addition, it also has significant potential to improve the chemical properties of degraded soil and subsequently improve plant growth and quality (Manzoor *et al.*, 2024).

Soil fertility is crucial for the sustainability of forage crops. In addition to its physical and chemical properties, soil fertility is also crucial for the sustainable role of the microbiome (Indriani *et al.*, 2016). Soil microbiomes as probiotics are very important for increasing soil fertility, which helps maintain soil health and also the quality of plant products. Microbiomes provide nutrients through natural processes such as nitrogen fixation, phosphorus solubilization and chemical synthesis that promote plant growth. Significant growth when the microbiome is inoculated with two or three microorganisms, not just one, called a consortium. Bacillus microorganisms, act as an effective biological control system. Some microorganisms are able to survive in various environments, expecting a synergistic way of working. They are also environmentally friendly and responsible for the sustainable availability of nutrients from natural sources (Muthusamy *et al.*, 2023). Mutualistic symbiosis of arbuscular mychorrizal fungi (AMF) occurs in a wide spectrum in higher plants.

Soil microbiomes as probiotics are essential for improving soil fertility, which helps maintain soil health and also the quality of plant products. The form of mutualistic symbiosis between fungi (myces) and roots (rhiza) of higher plants is known to provide a number of benefits to the host plant and the most important is its role in phosphorus absorption (Kumar *et al.*, 2012). Therefore, the future of sustainable crop production requires the use of alternative strategies that can increase crop yields in an environmentally friendly manner. Soil microbiomes are found in the rhizosphere (area around the roots) which can fix nitrogen, dissolve phosphorus and other organic macro and micro nutrients so that they become available to plants.

The coming years for microbiomes as plant probiotics in the field of research are expected to gain many benefits. Agricultural productivity is facing increasing pressures from urbanization, climate change and land use, threatening food availability. The use of plant probiotics offers a mechanism to reduce environmental stress including saline soils, acidic soils, heavy metal contamination, thereby increasing plant productivity. Some changes are macro, such as increased biomass and others also occur at the micro level that can accumulate unnoticed. The next challenge is to align the balance between the existing plant and soil microbiomes with the introduced plant probiotic microbiomes.

Microbial consortia are two or more interacting microorganisms involved, additive or synergistic results can be expected. The beneficial mechanisms of plant growth stimulation (*i.e.*, increased nutrient availability, phytohormone modulation, biocontrol, biotic and abiotic stress tolerance) are provided by different microbiomes in the rhizosphere, such as bacteria (Lactobacillus) and fungi (Trichoderma and Mycorrhiza).

Expanding the use of different microbial consortia, as well as increasing research on the different mixtures of microorganisms facilitate the best and most consistent results in the field. In this study, organic materials will be used and a probiotic microbiome consisting of the genera lactobacillus and saccharomyces, LAB (lactic acid bacteria) and mycofer (a consortium consisting of four mycorrhizal species: Glomus manihotis, Glomus etunicatum, Gigaspora margarita and Acaulospora sp).

MATERIALS AND METHODS

Research on the effect of various microbiomes as probiotics on thinning results (35 DAP) of corn plants [*Zea mays* (L.) Saccharata] on growth and production and quality of forage was conducted at the ciparanje research land and the animal feed crops laboratory, faculty of animal husbandry, padjadjaran university in Indonesia from August 2023 to February 2024. Starting from planting 2 corn seeds per hole, after 35 DAP, one of the plants in the hole was removed and named the thinning result which then became the object of research.

This study used an experimental method with a completely randomized design (CRD) consisting of 3 treatments of microbiome types and 6 replications. So there are 18 experimental units with treatment arrangements J1: aplication of probiotics lactobacillus + saccharomyces, J2: aplication of probiotics mycofer (*Glomus etunicatum*, *Glomus manihotis*, *Gigaspora margarita*, *Acaulospora* sp), J3: aplication of a combination (Lactobacillus + Saccharomyces) + BAL + mycofer. The aplication of inorganic fertilizers was only given half of the recommended amount given for all treatments (excluding treatment).

Observed variables

The variables observed in this study were plant growth (plant height). Production of plant thinning results (fresh weight and dry weight) and the quality of forage from thinning (crude protein, phosphorus, calcium).

RESULTS AND DISCUSSION

Plant height is one of the important factors in determining the biomass of forage. At 35 DAP plant height from the thinning results of baby-corn producing corn plants are presented in the following table:

Table 1 showed the high yields of plants for all treatments and ages of corn plants were not significantly different (the same) from each other, so the provision of Mycover was efficient. Mycover is able to increase the absorption of water and phosphorus and other essential minerals from the soil, thereby increasing the rate of plant growth (Wahab *et al.*, 2023). Saccharomyces plays a role in providing nutrients, phytohormones and dissolving phosphorus and zinc so that they are easily absorbed by plants (Vargas *et al.*, 2024). In addition, BAL and Lactobacillus can increase the availability of nutrients (Kang *et al.*, 2015), provide phytohormones IAA (indole acetic-acid), siderophores and phosphates (Shrestha *et al.*, 2014), as well as dissolve and increase the absorption of minerals (Amprayna *et al.*, 2016) so as to support plant growth. In this study, the combination of Lactobacillus + Saccharomyces, BAL and mycover (J3) gave a significantly lower difference compared to the Lactobacillus + Saccharomyces (J1) or mycover alone (J2) treatment at 35 DAP. In order to interact with plants, probiotic microbes need to colonize and compete with the natural microflora of corn plants. Their ability to colonize corn plants varies greatly depending on the ability of the microbial strain (Lorch *et al.*, 2024). The use of a consortium is considered more beneficial because it is more effective in colonizing the rhizosphere when compared to using only one type of microbe (Liu *et al.*, 2023). Mycover is a consortium of 4 single species that is very efficient in terms of economy because it is only given once to corn plants and is always there as long as there are still plants. In land where the plants have been harvested, there are still mycorrhizae in the form of spores and they can only be effective in inoculating the next plant in about 6 months. According to Indriani *et al.* (2019), mycorrhizae aid in the absorption of macro and micro nutrients and water, especially phosphate. Phosphate is commonly found in rocks and living organisms in the soil, so without additional phosphate to the soil, it is sufficient for plant growth for short time.

Table 2 indicated the results of fresh weight and dry weight for all treatments were not significantly different (the same) from each other, so the aplication of mycover was efficient. The interaction between plants and probiotics is mutual, namely probiotics play a role in increasing nutrient absorption and plant resistance while plants provide a carbon source for probiotic microbes (Wahab *et al.*, 2023). This can affect carbon allocation in plants and plant biomass (Holland *et al.*, 2019). Traits with moderate to high heritability and substantial genetic progress such as plant height and plant yields in the form of forage are selection criteria in breeding programs for forages that allow genetic improvement for biomass yields (Naharudin,

Table 1: Average plant height resulting from thinning (35 DAP) of corn plants in microbiome supplementation treatment as probiotics in corn plants [*Zea mays* (L.) Saccharata] as orage.

Treatment	Plant height (cm)				
	7 DAP	14 DAP	21 DAP	28 DAP	35 DAP
J1	13.32a	25.33a	42.08a	55.72a	74.03a
J2	12.70a	26.87a	41.42a	56.17a	70.40a
J3	12.57a	26.18a	37.00a	48.28a	59.00b

Notes: J1: Microbiome probiotic Lactobacillus + Saccharomyces. J2: Microbiome probiotic mycofer (*Glomus etunicatum, Glomus manihotis, Gigaspora margarita, Acaulospora sp*). J3: Microbiome combinations Lactobacillus + Saccharomyces, BAL dan mycover.

Table 2: Average fresh weight and dry weight production results of thinning (35 DAP) of corn plants in microbiome supplementation treatment as probiotics in corn plants [*Zea mays* (L.) Saccharata] as green forage.

Treatments	Fresh weight (g)	Dry weight (g)
J1	30.56a	3.57a
J2	30.94a	4.90a
J3	15.18a	2.17a

Notes: J1: Microbiome probiotic Lactobacillus + Saccharomyces. J2: Microbiome probiotic mycofer (*Glomus etunicatum, Glomus manihotis, Gigaspora margarita, Acaulospora sp*). J3: Microbiome combinations Lactobacillus + Saccharomyces, BAL dan mycover.

Table 3: Average crude protein, phosphorus and calcium results of thinning (35 DAP) in microbiome supplementation treatment as probiotics in corn plants [*Zea mays* (L.) Saccharata] as green forage.

Treatment	Crude protein (%)	Phosphor (%)	Calsium (%)
J1	16.75b	0.297a	0.445a
J2	17.16ab	0.293ab	0.423ab
J3	17.998a	0.273b	0.393b

Notes: J1: Microbiome probiotic Lactobacillus + Saccharomyces. J2: Microbiome probiotic mycofer (*Glomus etunicatum, Glomus manihotis, Gigaspora margarita, Acaulospora sp*). J3: Microbiome combinations Lactobacillus + Saccharomyces, BAL dan mycover.

et al., 2021). This causes forage yields not to be influenced by the provision of various types of microbiomes but rather genetic factors through selection to obtain various superior varieties, one of which is the bonanza variety.

Table 3 showed Crude Protein of corn forage J3 (17.998%) in the aplication of a combination of Lactobacillus + Saccharomyces, BAL and Mycover, was not significantly different from J2 (17.160%) in the aplication of Mycover, but was significantly higher than J1 (16.75%) in the aplication of a combination of Lactobacillus + Saccharomyces. Aplication of Mycover (J2) was more efficient than aplication of a combination of Lactobacillus + Saccharomyces, BAL and Mycover. The use of mycover in several studies also showed an increase in total protein which plays a role in increasing plant resistance to environmental stress (Wahab *et al.*, 2023). The study (Boutasknit *et al.*, 2020) showed that the use of mycorrhiza was able to significantly increase the protein content of locust bean plants (Ceratonia silique). Meanwhile (Garg and Singh, 2018) used Rhizophagus irregularis mycorrhiza which was able to increase proline biosynthesis in pigeon pea plants [Cajanus cajan (L.) Millsp.].

Along with the crude protein content with the most efficient provision of Mycover, the results for Phosphorus were J2 (0.293%) and calcium (0.423%). The advantage of providing mycover compared to Lactobacillus + Saccharomyces and BAL is that it helps plants absorb macronutrients, especially P and micronutrients and water. This can happen because mycover has a mycelium with a smaller size than plant roots, which increases its ability to penetrate large soil particles such as mineral crystals, aggregates and organic matter and is able to produce enzymes that can cause phosphate that was previously inaccessible to dissolve and can be absorbed by plants (Pepe *et al.*, 2022).

CONCLUSION

Based on the results of research on various microbiomes as probiotics in the thinning (35 DAP) of corn plants (*Zea mays* Saccharata L.) can affect plant height and quality of thinning forage and the same results for fresh weight and dry weight of thinning forage. Plant height of the (35 DAP) mycofer treatment produces a plant height of 70.40 cm, crude protein of 17.16%, Phosphorus of 0.293% and calcium of 0.423% as the best results compared to others.

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Conflict of interest

All authors declare that they have no conflict of interest.

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