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Symbiotic Efficiency of Arbuscular Mycorrhizal Fungi on the Growth Dynamics and Root Colonization of Waxy CORN (*Zea mays* L. var. ceratina)

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ABSTRACT. One of the important food crops in Gorontalo is corn (Zea mays L. var. ceratina), and root health is very important in determining its growth and productivity. This study aims to determine the root shoot-root ratio and root infection of waxy corn plants given arbuscular mycorrhizal fungi as biostimulants. This study was conducted from July to December 2025 using a completely randomized block design (RBD) with four different doses mycorrhizal arbuscular: 0, 10, 20, 30 g /polybag, which were replicated three times and observed periodically over 42 days after planting (DAP). The variables observed were root shoot-root ratio and root infection of plants at 14, 21, 28, 35, and 42 days after planting. The results showed that the application of mycorrhizae with different doses resulted in a root shoot ratio that increased in line with the increasing age of the plant. In contrast, it was interesting to find that in plant root infections, mycorrhizal application appeared to increase until the plants were 28 days after planting, after which it declined. This finding suggests that arbuscular mycorrhizal inoculation given to plants at different doses can impact root health, as indicated by the ratio of root decay to root infection. The symbiosis between mycorrhizae and plants is crucial for the growth of waxy corn.

Keywords : *Arbuscular mycorrhizal; Gorontalo; root infection; shoot-root ratio; waxy corn*

INTRODUCTION

Global challenges triggered by climate change and the depletion of essential nutrient reserves, particularly phosphorus (P), have become a real threat to the stability of the world's food production systems. Water scarcity and soil fertility degradation directly impact the reduction of nutrient absorption capacity by plants, which in turn significantly suppresses crop yields (Abd El-Fattah et al., 2023). In the landscape of modern agriculture, cultivation practices that heavily rely on synthetic inorganic fertilizers have proven to be ecologically and economically unsustainable. Therefore, manipulating the rhizosphere microbiome, particularly through the utilization of Arbuscular Mycorrhizal Fungi (AMF), is increasingly recognized as the most promising biological strategy to enhance nutrient availability, optimize water use efficiency, and build agroecosystem resilience (Mäkelä et al., 2020; Ou-Zine et al., 2022).

Indonesia, specifically Gorontalo Province, possesses a remarkable wealth of local maize germplasm, such as the Pulut (Binthe Pulo), Momala, Siropu, and Doti varieties. These local varieties are known to have unique morphological adaptation advantages, high nutritional value, and close genetic kinship (Kandowangko et al., 2025; Tadidik et al., 2025). These local maize varieties play an important role in traditional agro-management practices as well as local food utilization; for instance, the Momala variety is rich in anthocyanins, while Pulut has a very high amylopectin content (Kandowangko et al., 2020). However, these varieties possess a rapid vegetative growth rate that demands optimal nutrient and water availability. Under drought stress conditions, local maize such as Pulut and Siropu often exhibit a decline in growth parameters such as root dry weight and plant height (Latif et al., 2023).

These plants rely heavily on their root architecture and health to meet these physiological demands. Unfortunately, in marginal quality soils or phosphorus-deficient lands, maize roots often fail to extract nutrients optimally due to the very low mobility of phosphates in the soil (Tammam et al., 2022). This is where the mutualistic symbiosis with AMF plays its vital role.

Arbuscular Mycorrhizal Fungi (AMF) form obligate symbiotic associations with over 80% of terrestrial plant species, including maize. These fungi act as a functional extension of the plant's root system through the formation of an extensive extraradical mycelial network (Truong et al., 2025). This hyphal network has a much smaller diameter compared to root hairs, allowing it to penetrate soil micropores that are inaccessible to plant roots. Through this mechanism, AMF facilitates increased uptake of macronutrients (especially P and N) and micronutrients, while concurrently enhancing plant tolerance to abiotic stresses such as drought and salinity (Gao et al., 2023).

Although the potential of AMF is well-documented, the dynamics of this symbiosis are highly complex and do not always result in linearly positive growth effects. The success of the interaction between AMF and maize is influenced by various factors, including the host species, the identity of the fungal strain, soil legacy conditions, and the applied inoculant dose (Dias et al., 2018; Haro et al., 2018). Inoculation with an inappropriate dose can cause carbon competition between the fungus and the host plant, leading to growth depression (mycorrhizal parasitism), or conversely, fail to form adequate colonization due to competitive pressure from the native soil microbiome (Sefrila et al., 2023).

A primary indicator of successful symbiosis and plant adaptation to its environment is allometric growth dynamics, which can be measured via the shoot-to-root ratio. This ratio reflects the plant's carbon assimilate partitioning strategy in response to resource availability above and below the soil surface. Furthermore, monitoring the level of root colonization at various growth phases is critical to understanding when the functional transition from the mycelial invasion phase to the nutrient exchange phase occurs. However, empirical data regarding the effect of local inoculant dose variations on the shoot-to-root ratio profile of local maize across a growth time series remain very limited.

The novelty of this research lies in its temporal evaluation approach linking allometric growth dynamics (shoot-to-root ratio) with the colonization kinetics of indigenous Arbuscular Mycorrhizal Fungi (AMF) in local waxy corn (Zea mays L. var. ceratina). Previous studies have predominantly focused on the basic morphological, anatomical, and nutritional characterizations of local Gorontalo maize (Ahaya et al., 2025; Kandowangko et al., 2025), or on their general physiological responses—such as proline and abscisic acid (ABA) accumulation—under drought stress (Kandowangko et al., 2009; Latif et al., 2023). However, no comprehensive study has mapped the exact equilibrium point (sweet spot) of the symbiotic interaction between local microbiomes and the root architecture of Gorontalo waxy corn across a developmental time series (14 to 42 Days After Planting).

This study bridges that gap by not only measuring infection success but also quantifying how specific inoculum doses (e.g., 20 g/polybag) actively modify carbon assimilate partitioning to prevent the top-heavy canopy phenomenon. The integration of indigenous mycorrhizal strains with an endemic maize variety offers new, high-precision agronomic insights. This is highly crucial as a foundation for climate-resilient agro-management and serves as a functional conservation effort for local germplasm whose microbiome potential has previously remained underexplored. Based on these knowledge gaps, this study was designed to systematically evaluate the dynamics of the shoot-to-root ratio and root infection percentage of local maize following the application of various mycorrhizal inoculant doses.

MATERIALS AND METHODS

Research Location and Timeline

Research activities were executed at dual locations, centered in the Biology Laboratory under the auspices of the Faculty of Mathematics and Natural Sciences at Gorontalo State University. Corn planting was carried out in an experimental garden using polybags. The location is in Wumialo Village, Kota Tengah District, Gorontalo City, Gorontalo Province, Indonesia. The research period was 6 months, from July to December 2025.

Equipment and Materials

The equipment used included: an autoclave, laminar air flow meter, spectrophotometer, incubator, drying oven, analytical balance, drying oven, shovel, scissors, labels, ruler, measuring tape, petri dish, micropipette, loop needle, centrifuge, Bunsen burner, test tube, glass funnel, cuvette, mortar, stationery, and 35 x 35 cm plastic bags.

Materials used: waxy corn seeds (*Zea mays* var. ceratina) and biostimulants mycorrhizal arbuscula containing the genus *Glomus, Acaulospora, Gigaspora,* and *Scutellospora,* soil as a planting medium, soil, aquades, 70% alcohol, 10% KOH solution, glacial acetic acid, and Fuchsin acid solution for mycorrhizal structure staining. The tools used include analytical scales, drying ovens, light microscopes, measuring cups, beaker cups, tweezers, razors, scissors, hotplates, preparation glass, bunsen, and cameras for documentation

Research Procedures

Preparation of Plant Materials and Experimental Soil Media

The plant materials used were local Gorontalo corn seeds, waxy corn (*Zea mays* L. var. ceratina). The soil media used was garden soil. Prior to the experiment, an initial soil chemical analysis was conducted using a composite soil sample. The initial soil sample was analyzed at the Gorontalo Agricultural Research and Modernization Agency (BRMP) laboratory. The analysis results indicated high phosphorus content, low potassium content, slightly acidic pH, and low organic carbon content.

Experimental Design

This study used an experimental method with a randomized block design with four AMF treatments: PM0 (control without inoculation); PM_1 (10 g AMF/polybag); PM_2 (20 g/per polybag); and PM_3 (30 g per polybag); which were replicated three times, resulting in 12 experimental units, and plants were observed at 14, 21, 28, 35, and 42 days after planting. This resulted in 60 experimental units. Each polybag was filled with 10 kg of soil. The soil was then dug to a depth of 5–7 cm. Mycorrhizal inoculant was applied according to the treatment dosage, and three waxy corn seeds were planted per polybag. Plants were maintained until they reached the maximum vegetative stage with regular watering and weed control.

Shoot-to-Root Ratio

Determined through destructive harvesting. The plants were separated into above-ground parts (shoot: stem and leaves) and below-ground parts (roots). The samples were dried in a constant-temperature oven until they reached absolute dry weight. The ratio was calculated by dividing the shoot dry weight by the root dry weight.

Observation of the shoot to root ratio was carried out in the vegetative phase, namely at the age of 14, 21, 28, 35 and 42 days after planting in previous studies (Rusmana, 2017). The shoot-root ratio or root canopy ratio value is determined by comparing the dry weight value of the canopy with the dry weight of the root.

Root Infection Percentage

Evaluated using the root staining method. Fine root segments were cleared with KOH, acidified, and stained before being observed under a high-magnification microscope. The presence of typical arbuscular mycorrhizal structures, such as internal hyphae, vesicles (lipid storage organs), and arbuscules (primary sites of nutrient exchange), was quantified using a modified grid-line intersect method to determine the colonization percentage relative to the total observed root length (Sefrila et al., 2023; Truong et al., 2025).

Data Analysis

The final data were analyzed descriptively by calculating the mean value and standard deviation of each treatment.

RESULTS AND DISCUSSION

Allometric Growth Dynamics and Shoot-to-Root Ratio

The biomass allocation profile in waxy corn, represented by the shoot-to-root ratio (shoot dry weight divided by root dry weight), exhibited a highly dynamic response to the presence and dose of Arbuscular Mycorrhizal Fungi (AMF) inoculant. Based on the presented data (Table 1), a clear pattern of divergence emerged between the control plants (PM0) and the inoculated plants (PM1, PM2, PM3) as the plants aged. During the initial observation phase (14 DAP and 21 DAP), all treatments, both control and mycorrhizal, showed relatively uniform shoot-to-root ratios, ranging from 2.23 to 3.89. This figure reflects the seedling phase where nutrient reserves from the seed endosperm still dominate growth support, and carbon investment is proportionally focused on the early formation of leaves and primary roots.

However, entering the exponential growth phase (28 DAP to 35 DAP), the disparity in assimilate partitioning became dramatically apparent. In the control treatment (PM0), the shoot-to-root ratio spiked sharply to 6.81 at 35 DAP and reached its peak at 10.24 at 42 DAP. This extreme ratio in PM0 indicates an allometric anomaly known as the *top-heavy* phenomenon. In the absence of a biological assistant like mycorrhiza, the maize plant is forced to allocate almost all of its photosynthetic products to form canopy organs (leaves and stems) in an effort to maximize light capture. Unfortunately, this massive canopy growth is not balanced by adequate root development. According to Abd El-Fattah et al. (2023), maize plants with an excessively high shoot-to-root ratio are highly susceptible to abiotic stresses; they lack sufficient root exploration capacity to support the transpirational water demands.

Table 1. Average Ratio of waxy corn plant root extinction at each observation interval

Treatment	Average Shoot Root Ratio				
	14	21	28	35	42
PMO	2,87	3,40	3,40	6,81	10,24
PM1	2,23	2,90	3,83	4,69	7,85
PM2	3,89	3,03	4,05	4,66	5,06
PM3	3,68	2,57	2,98	9,02	4,11

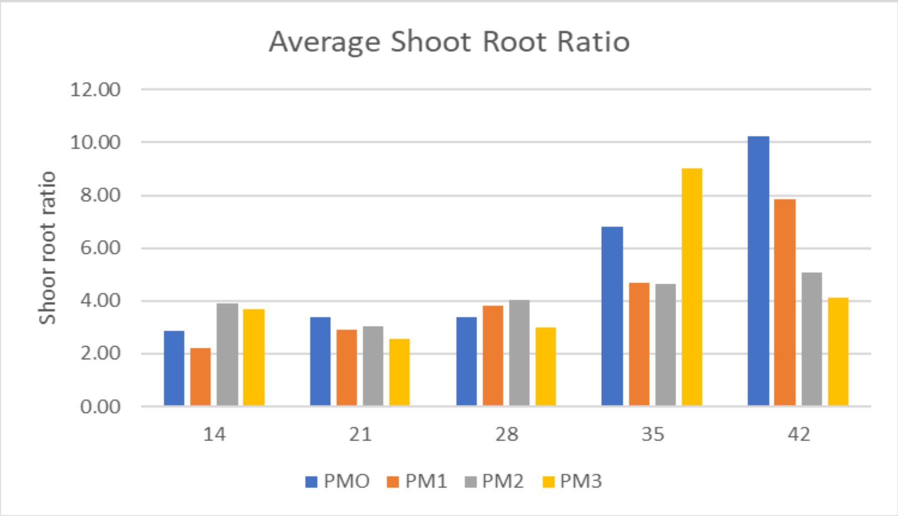


Figure 1. Shoot Root Ratio Graph

Conversely, AMF inoculation fundamentally modified the plant's architectural program. At 42 DAP, treatments with moderate to high mycorrhizal doses significantly suppressed the shoot-to-root ratio. PM2 (20 g) recorded a ratio of 5.06, while PM3 (30 g) produced the lowest ratio of 4.11. This reduction in the ratio is not caused by stunted shoot growth, but is rather tangible evidence of an exponential expansion of root mass. Drought stress in marginal lands generally drastically reduces root volume and dry weight (Latif et al., 2023). However, AMF acts as a robust carbon "sink" that stimulates lateral root proliferation and fine root modification. This root expansion is anatomically supported by the development of denser vascular tissues (xylem and phloem) with thicker cell walls in local maize plants, as evidenced by the observation of the anatomical structure of the stems and leaves of Pulut and Momala maize at 30-40 DAP (Ahaya et al., 2025). This more robust vascular network is crucial to supporting massive hyphal colonization and maintaining smooth nutrient transportation.

The decrease in the shoot-to-root ratio in the PM3 treatment (30 g) to 4.11 at the end of the observation (42 DAP), although appearing as an improvement in root architecture, raises a physiological discourse related to the symbiotic cost-benefit. The high inoculum intake in PM3 may trigger a condition known as *carbon drain*, where shoot biomass formation is slightly withheld to "feed" the dense fungal population in the subterranean ecosystem (Dias et al., 2018). Therefore, the ratio of 5.06 in the PM2 treatment (20 g) is considered a reflection of a more ideal agronomic balance, representing a truly mutualistic symbiosis.

A (14 DAP) B (21 DAP) C (28 DAP) D (35 DAP) E (42 DAP)

Figure 2. Root shoot ratio of waxy corn (*Zea mays* L. Var. Caratina) in various mycorrhizal treatments (10 g; 15 g; 30 g and Control), at various observation intervals

Colonization Kinetics and Root Infection Percentage

The successful modification of plant architecture correlates directly with the progress of AMF colonization within the root cortex tissue. Based on the root infection percentage data (Figure 1), the symbiotic process does not occur instantaneously but through gradual phases of recognition, penetration, and proliferation. At 14 DAP and 21 DAP, the root infection rate across all inoculated treatments was at the initial stage (below 50%). This phase is a critical period where fungal hyphae guide themselves towards the root epidermis and begin penetrating the plant cell walls (Adeyemi et al., 2019).

Table 2. Average root infection of waxy corn plants at each observation interval

Treatment	Average root infection of waxy corn plants (%)				
	14 DAP	21 DAP	28 DAP	35 DAP	42 DAP
PM 0	0,00	0,00	0,00	0,10	0,20
PM 1	0,30	0,30	0,30	0,50	0,20
PM 2	0,50	0,20	0,80	0,30	0,10
PM 3	0,40	0,40	0,70	0,50	0,50

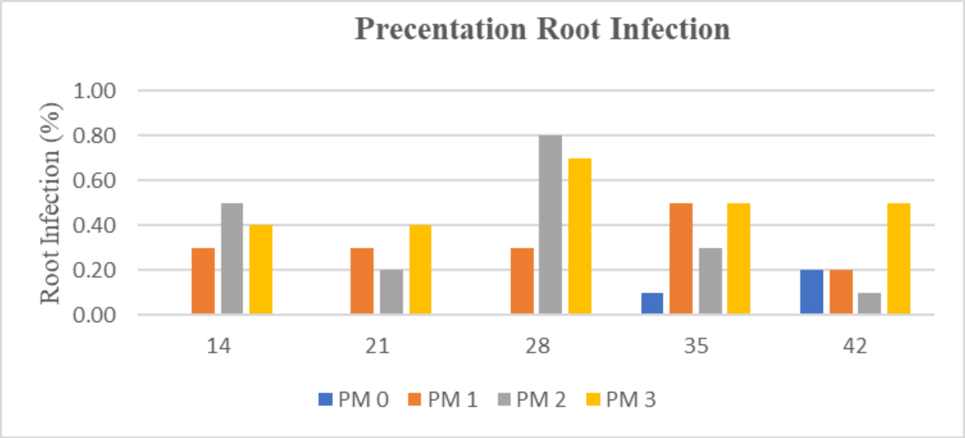


Figure 3. Root Infection Graph

The peak of colonization kinetics occurred at 28 DAP, representing the maximum vegetative phase in maize, just before the plant prepares to enter the floral initiation phase. At this point, the PM2 treatment (20 g/polybag) recorded a phenomenal surge in root infection, reaching 80%. This exceptionally high infection rate indicates that the maize root cortex has been replete with arbuscular structures and vesicles. Interestingly, the highest dose treatment PM3 (30 g) at 28 DAP "only" reached 70% infection. This phenomenon indicates a *density-dependent inhibition* effect or intra-specific competition among fungal propagules in competing for infection sites on the root epidermal surface (Sefrila et al., 2023).

Entering the 35 DAP and 42 DAP observations, an interesting trend shift occurred where the infection percentage tended to decline sharply in PM2, while PM3 and PM1 fluctuated stably around 50%. This decrease in infection percentage is a manifestation of the *biological dilution effect*. During this timeframe, maize focuses its growth on massive root volume expansion. The volume of newly produced roots far exceeds the penetration speed of the fungal hyphae to colonize them (Truong et al., 2025).

Delving deeper into physiological aspects, high colonization during this vegetative phase plays an important role in the plant's biochemical response to the environment. A study by Kandowangko et al. (2009) confirmed that AMF inoculation on maize plants experiencing drought stress can synergistically increase proline accumulation (as an osmoregulator retaining water in cells) while significantly reducing the level of the hormone Abscisic Acid (ABA) in the leaves. This reduction in ABA prevents premature stomatal closure. Anatomically, this adaptability aligns with changes in stomatal density and epidermal cell thickness in the leaves of local maize (such as the Momala variety) as the plant ages, which helps suppress transpiration while maintaining the photosynthetic rate (Ahaya et al., 2025). During the transition to the generative phase, the role of AMF shifts from tissue invasion to maintenance functionality of nutrient supply (Yadav et al., 2023).

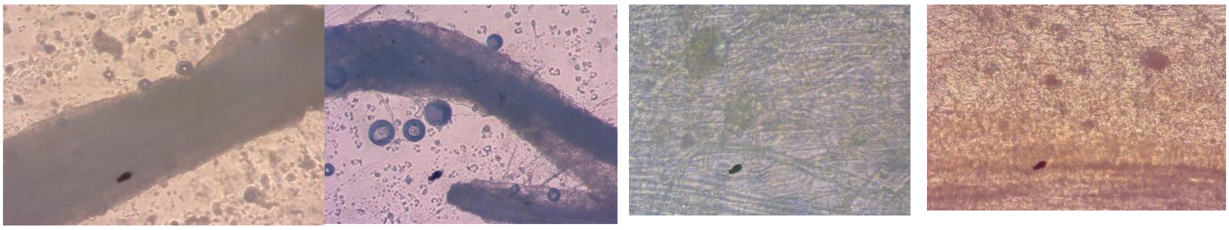
Symbiotic Efficiency and Ecological-Agronomic Implications

The integration of the shoot-to-root ratio data and the infection graph establishes the PM2 treatment (20 g dose) as the best equilibrium point (*the optimal symbiotic threshold*). This dose triggered the most aggressive colonization response during the critical period (80% at 28 DAP) and successfully formed a solid foundation for root architecture (shoot-to-root ratio 5.06 at 42 DAP). Mechanistically, AMF inoculants release acid and alkaline phosphatase enzymes into the soil which dissolve inorganic phosphates, literally "unlocking" nutrient vaults that are unavailable to roots without mycorrhizae (Tammam et al., 2022).

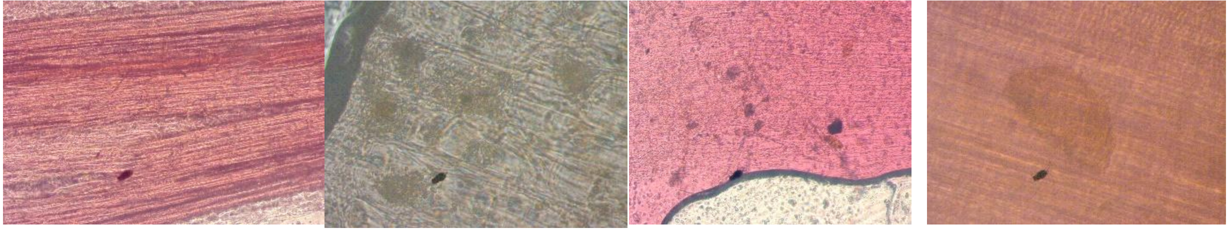
The success of this colonization is closely related to the ecological identity and genetic diversity of both the host and its microbes. Local Gorontalo maize, such as Binthe Pulo, Siropu, and Momala, morphologically share close kinship and have long adapted to local edaphic and microclimate conditions (Tadidik et al., 2025; Kandowangko et al., 2025). Indigenous mycorrhizal consortiums have proven to adapt better to specific environmental stresses in tropical soils compared to introduced commercial mycorrhizal strains (Haro et al., 2018). Moreover, the traditional agro-management practices of the local Gorontalo community, which maintain the *huyula* (mutual cooperation) planting system and minimize the use of synthetic chemicals, provide an excellent space for the preservation of the natural rhizosphere microbiome (Kandowangko et al., 2020).

AMF hyphae also secrete glomalin, an adhesive glycoprotein that functions to bind soil particles into stable macro-aggregates. Well-structured soil resulting from mycorrhizal glomalin improves porosity and water retention, facilitating the physical penetration of maize roots, which indirectly supports the expansion of the root system and lowers the shoot-to-root ratio. This synergy can be exponentially enhanced through the incorporation of organic amendments (phospho-compost or liquid organic fertilizer) that provide slow-release nutrients (El Gabardi et al., 2020; Ou-Zine et al., 2022).

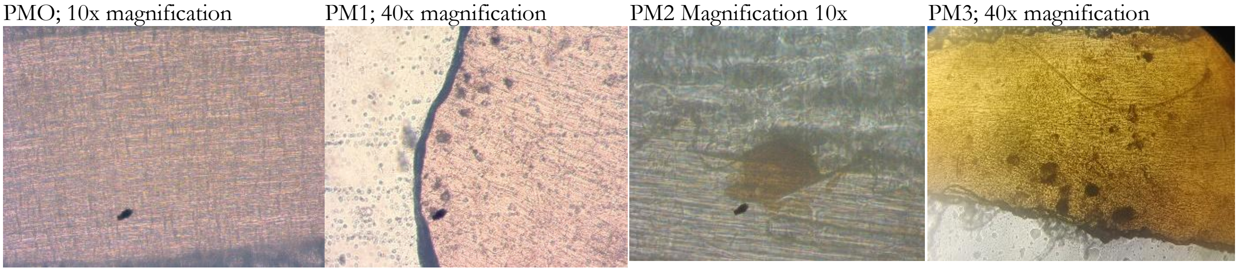
Overall, these findings provide precise agronomic guidance that AMF inoculation at a dose of 20 g is an integral component in future maize cultivation practices. This approach not only preserves superior local germplasm like Pulut and Momala but also transforms the maize architecture from *top-heavy* into a plant with a dominant root system. This ensures the efficient use of water and nutrients, increases productivity stability, and has the potential to massively cut the cost and residue of synthetic phosphorus fertilizer use in fields (Baltruschat et al., 2019; Zhang et al., 2018).



PMO; 10x magnification PM1; magnification 10x PM2; 40x magnification PM3; 10x magnification



PMO; 10x magnification PM1; 40x magnification PM2 Magnification 10x PM3; 40x magnification



PMO; 10x magnification PM1; 10x magnification PM2; 40x magnification PM3; Magnification10x

Figure 4. Plant Root Infections given Mycorrhizal arbuscula

CONCLUSION

Arbuscular Mycorrhizal Fungi (AMF) inoculation has proven to be more than just a nutrient provisioning agent; it acts as the primary architect that restructures biomass partitioning and growth dynamics in waxy corn (Zea mays L. var. ceratina). This study concludes that the application of AMF inoculant significantly suppresses the dominance of shoot growth and reallocates plant carbon investment toward the expansion of the root system, preventing the plant from the imbalanced architectural syndrome (top-heavy) observed in the control (PM0). Agronomically, the 20 g/polybag (PM2) dose was identified as the equilibrium point (optimal dose), capable of producing the highest peak of colonization infection at 80% during the critical phase of 28 DAP. This optimal colonization level successfully balanced the formation of vegetative organs with the availability of absorptive root infrastructure, which has the potential to maximize P uptake and agroecosystem resilience against extreme climate changes.

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