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Production of Arbuscular Mycorrhizal Fungi Inoculum at Various Cow Urine POC Concentrations Using Sorghum *(Sorghum bicolor* L. Moench) Host in Hydroponic System

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ABSTRACT. This study aimed to evaluate the production of arbuscular mycorrhizal fungi (AMF) inoculum at various concentrations of cattle urine-based liquid organic fertilizer (LOF) using sorghum (Sorghum bicolor L. Moench) as the host plant in a hydroponic system. The experiment was conducted using a Completely Randomized Design (CRD) with five treatments: PO (control), P1 (100% AB Mix), P2 (25 mL L-1 cattle urine LOF), P3 (50 mL L-1 cattle urine LOF), and P4 (75 mL L-1 cattle urine LOF). The observed parameters included the percentage of root colonization, spore density per 10 g of substrate, and host plant growth, as indicated by plant height and biomass. The results demonstrated that cattle urine concentration had a significant effect on the success of AMF symbiosis and spore production. Treatments PO (control without nutrient addition) and P2 (25 mL L-1 cattle urine LOF) produced the most effective outcomes, as indicated by the highest spore density and root colonization percentage compared to the other treatments. These findings suggest that low-nutrient conditions or low concentrations of cattle urine create an optimal environment for AMF proliferation. In conclusion, hydroponic production of AMF inoculum under minimal nutrient input or low doses of cattle urine represents an effective method for producing high-quality biofertilizer.

Keywords : *Arbuscular Mycorrhizal Fungi, Hydroponics, Inoculum, Sorghum, Cattle Urine.*

INTRODUCTION

The agricultural sector plays a crucial role in providing sufficient food for the population. As challenges intensify with advancing times, agriculture must not only ensure adequate food quantity but also meet continuously rising quality standards. One strategy to enhance crop quality involves adopting organic farming systems; however, limited organic fertilizer availability remains a primary constraint. Mycorrhizal biofertilizers represent a key organic option, comprising arbuscular mycorrhizal fungi as the active ingredient alongside carrier materials (Setyaningsih et al., 2023).

In 2019, the global market for arbuscular mycorrhizal fungi (AMF) biofertilizers reached USD 268.8 million, with projections indicating a 131.25% growth to approximately USD 621.6 million by 2025 (MIO, 2020). Despite this surging demand, large-scale production of AMF biofertilizers faces persistent technical hurdles. A primary constraint is the challenge of generating sufficient quantities of AMF inoculum (Mishra et al., 2018). AMF inoculum typically comprises spores intermixed with roots colonized by the fungi (Nurbaity, 2019). Key limitations to its scalability include extended production timelines (up to three months), dependence on manual irrigation and nutrient addition, and the limited output of traditional pot culture systems (Jeffries et al., 2003).

Sorghum bicolor serves as an ideal host plant for producing arbuscular mycorrhizal fungi (AMF) biofertilizers. This crop readily forms symbiotic associations with AMF, which enhance uptake of key nutrients such as phosphorus (P) and nitrogen (N), while also boosting

water use efficiency. Sorghum is notably tolerant of suboptimal soil conditions, and its partnership with AMF further bolsters resilience to harsh environments, including nutrient-poor soils (Fitria et al., 2022). The nutrient regime supporting host plant growth critically influences AMF inoculum propagation. Hydroponic systems with varying phosphorus concentrations yield significant differences in spore abundance. However, these treatments elicit no significant variation in mycorrhizal root colonization percentages in sorghum. Nonetheless, colonization levels in sorghum roots consistently exceed 65%, qualifying as high. Low phosphorus doses (20 and 40 ppm) notably achieve spore productivity surpassing standard thresholds for mycorrhizal biofertilizers (Nurbaity, 2019).

Liquid organic fertilizers derived from cow urine serve as a high-phosphorus (P) nutrient source. These fertilizers arise from the decomposition of natural materials, such as organic waste and biological by-products, and are available in solid or liquid forms; cow urine is particularly noteworthy for its ready availability and nutrient richness. It contains elevated levels of nitrogen (N), phosphorus (P), potassium (K), and calcium (Ca), while enhancing plant resistance to diseases (Phrimantoro, 2002, as cited in Rizki, 2014). Laboratory analysis reveals distinct changes in cow urine properties pre- and post-fermentation (Affandi, 2008). Before fermentation, it exhibits a pH of 7.2, with 1.1% N, 0.5% P, 1.5% K, 1.1% Ca, yellow coloration, and strong pungent odor. Post-fermentation, the pH rises to 8.7, nutrient levels increase to 2.7% N, 2.4% P, 3.8% K, and 5.8% Ca, accompanied by a blackish hue and reduced odor intensity.

In addition to nutrient availability, the AMF production system profoundly influences spore growth. Three primary systems are currently employed: substrate-based, substrate-free, and in vitro production methods. Substrate-free systems stand out for inoculum production, generating healthier AMF propagules that can be applied directly without further refinement. A prime example is the ebb-and-flow hydroponic system, which leverages periodic flooding and draining of nutrient solutions. The flooding phase inundates roots with nutrients, while the draining phase facilitates solution recession and root zone re-aeration. This cyclic mechanism optimizes aeration in the growth medium, providing superior oxygen availability relative to other systems (Delya, 2014).

Given the rising demand for arbuscular mycorrhizal fungi (AMF) biofertilizers and persistent production challenges, this study investigates the role of liquid cow urine fertilizer in enhancing AMF output. Specifically, it examines how varying concentrations of this fertilizer influence AMF spore proliferation, providing practical guidelines for optimizing mycorrhizal biofertilizer production to be more effective, cost-efficient, and farmer-independent.

MATERIALS AND METHODS

This study was conducted at the Basic Agrotechnology Laboratory and Applied Agrotechnology Laboratory, Faculty of Agriculture and Fisheries, Muhammadiyah University of Purwokerto, as well as Greenhouse Experimental Plot 2 at the same institution. Research activities spanned February to April 2024.

Materials and Methods

The equipment utilized in this study included a hydroponic installation, automatic switch, oven, seedling trays, knives, Petri dishes, Erlenmeyer flasks, beakers, measuring cylinders, test tubes with racks, analytical digital balance, microscope, glass slides and coverslips, gauze cloth, string, ruler, trays, filter paper, sieves (425 µm, 212 µm, 106 µm, and 60 µm), soil sterilizer, scissors, stereo microscope, and light microscope.

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The materials employed in this study comprised mycorrhizal biofertilizer (Indo Biotech Agro), zeolite, rice husk charcoal, water, liquid cow urine organic fertilizer, sorghum seeds, botanical pesticides, 10% KOH solution, 5% vinegar solution, glycerin solution (30 ml), lactic acid solution (30 ml), lactoglycerol, distilled water (aquades), and Parker Quick ink (5 ml).

Research Implementation

The research commenced with assessing the arbuscular mycorrhizal fungi (AMF) inoculum starter to verify spore density via the wet sieving method. Concurrently, an ebb-and-flow hydroponic system was assembled and modified with an automatic timer outlet in the Muhammadiyah University of Purwokerto greenhouse. The draining (off) phase was programmed for 15 minutes twice daily at 09:00 and 15:00 WIB, following Nurbaity et al. (2019). Prior to use, the zeolite growing medium was sterilized in a soil sterilizer at 121°C for 30 minutes to eliminate contaminating microorganisms and minimize competition against mycorrhizae.

Cultivation commenced with the germination of sorghum seeds pre-soaked for 24 hours. Seeds were sown in seedling trays amended with mycorrhizal inoculum at 10% of the medium weight (equivalent to 200 g per 2 kg medium). At two weeks of age, seedlings were transplanted into 5-cm-diameter net pots filled with a zeolite and rice husk charcoal mixture (3:1 ratio). Initial nutrient application occurred during transplanting by dissolving cow urine POC into the nutrient reservoir at specified treatment dosages. The nutrient solution was fully refreshed weekly to sustain nutrient availability for plant growth.

During the six-week growth period in the hydroponic system, plant health was maintained through integrated pest management employing both botanical pesticides and mechanical techniques. At the final stage, a drying or stress treatment was imposed by withholding nutrients for 14 days to stimulate spore formation. The experiment concluded with harvesting, entailing separation of shoots and roots; the growing medium containing AMF-colonized roots was then collected and stored in a dry location for use as produced inoculum source.

RESULTS AND DISCUSSION

DMRT analysis results at the 5% significance level, illustrating the effects of cow urine POC concentrations on host plant growth and mycorrhizal spore production in sorghum, are presented in Table 1.

Table 1. Effects of cow urine POC concentrations on host plant growth and mycorrhizal spore production in sorghum.

Treatments	Observed Variables						
	SC	RI	PH	LN	LA	FW	DW
P0	219,2d	76b	9,04a	6a	67,5a	3,396a	0,264a
P1	27a	8a	11,74c	7,6a	133,875c	13,7c	1,444b
P2	211,4d	68b	9,9a	6,2a	82,21875a	5,86b	0,362a
P3	123,8c	60b	10,75c	5,6a	107,875c	7,27b	0,428a
P4	72,4b	32a	11,9c	6,6a	106,6865c	12,86c	1,162b
	*	*	*	tn	*	*	*

Note: SC (Spore count), RI (Root infection), PH (Plant height), LN (Leaf number), LA (Leaf area), FW (Fresh weight), DW (Dry weight). Means followed by different letters within columns indicate significant differences according to DMRT test at 5% level (initial spore application ±10^6).

Spore Count

Table 1 indicates that treatments P0 and P2 exhibited statistically similar results with no significant difference, yet these treatments achieved the highest spore counts compared to all other treatments. Both P0 and P2 demonstrated a twofold increase in spore numbers from the initial application, rising from 106 spores to 211–219 spores per sample. The superior spore production in P0 (host plants receiving no nutrient supplementation) and P2 (host plants treated with the lowest cow urine concentration of 25 cc/L relative to other treatments) can be attributed to the nutritional stress imposed on the sorghum host plants, which optimally stimulated AMF sporulation.

The relationship between nutrient availability particularly phosphorus in the growing medium and arbuscular mycorrhizal fungi (AMF) proliferation, including sporulation, has been extensively investigated. Numerous studies demonstrate that lower total phosphorus levels in soil or growth media correlate with higher AMF spore densities (Saputra et al., 2015). Under nutrient stress, especially phosphorus deficiency, host plants intensify chemical signaling to stimulate AMF colonization and development, as they become increasingly reliant on these fungi to access scarce essential nutrients. This heightened dependence fosters stronger symbiotic interactions, ultimately prompting AMF to enhance sporulation as an adaptive strategy for survival in stressful environments (Diputra et al., 2018; Smith & Read, 2010).

AMF derives carbon (sugars) from its host plant. Under nutrient deficiency, the host allocates greater amounts of carbon to its symbiotic partner (AMF) to optimize uptake of scarce nutrients. This augmented carbon supply signals to AMF sufficient resources for propagule production specifically sporulation (Igiehon & Babalola, 2017).

AMF spore production frequently surges under conditions deemed unfavorable by the fungus, such as drought stress or in this case nutrient limitation. Enhanced sporulation under stress serves as a key survival and dispersal strategy for AMF (Diputra et al., 2018). Treatment P0 (no nutrients) clearly induced nutrient stress, while P2 with the lowest cow urine POC concentration (25 cc/L) likewise generated markedly restricted nutrient availability compared to P3 and P4 treatments, eliciting a comparable sporulation response.

Root Infection

Table 1 reveals that treatments P0, P2, and P3 exhibited the highest root infection levels, significantly surpassing other treatments. Root infection by AMF generally correlated positively with spore production, except in P3. Greater infected root mass typically yields higher spore counts; however, P3 represents an exception attributable to AMF acting as a carbon sink. A carbon sink refers to a system or process natural or artificial that sequesters more atmospheric CO_2 than it emits. Here, AMF, as obligate biotrophs incapable of photosynthesis, excessively draws carbon (as photosynthate sugars) from the host. Elevated infection establishes extensive hyphal networks (arbuscules and vesicles) intra- and extra-radically, demanding continuous sugar supply from the plant. This intensive colonization functions as a substantial carbon sink, diverting plant biomass without strong stress signals that would otherwise trigger prolific sporulation (Smith, 2008).

Root infection signifies successful mycorrhizal biofertilizer inoculation on host plant roots used for propagating arbuscular mycorrhizal fungi (AMF) inoculum. Upon root infection by AMF, mutualistic symbiosis develops between the plant and fungus, facilitating mycorrhizal growth and development that yields new spores. In the control treatment (P0: mycorrhiza without nutrients), root infection achieved optimal levels. This aligns with AMF symbiosis ecology, where environmental nutrient deficiencies particularly phosphorus (P) prompt responsive sorghum hosts (at 42 days after sowing) to actively channel carbohydrates to roots, exchanging them for P acquired by AMF. Such nutrient scarcity generates potent

signals that reinforce symbiotic associations. Conversely, treatment P1 (mycorrhiza + ABMix 40 ppm) exhibited significantly suppressed infection. Complete inorganic fertilizers like ABMix supply readily soluble, absorbable P at adequate concentrations (40 ppm), suppressing root-emitted biological signals. This diminishes plant reliance on AMF a phenomenon termed phosphorus-induced colonization inhibition (Ishii, 2004).

Meanwhile, treatments with liquid cow urine organic fertilizer (POC; P2, P3, P4) exhibited distinct dynamics. Root infection in POC groups (P2, P3) generally exceeded P1 levels but remained slightly below P0. Phosphorus in POC exists in organic forms requiring microbial mineralization for gradual release, thus exerting less suppressive pressure on symbiosis than inorganic fertilizers (Pranata et al., 2023). Organic components in POC likely enhance rhizosphere conditions, synergistically promoting root health and AMF activity. However, escalating POC dosage to P4 (75 cc/L) re-suppressed infection. Excessively high nitrogen and potassium levels likely accelerated vegetative growth, curtailing carbon allocation to AMF. Additionally, potential phytotoxicity or elevated salt concentrations at extreme doses may inhibit external hyphal elongation, compromising colonization efficacy (Wang et al., 2022).

In the context of spore production, an indicator of propagules for subsequent cropping cycles a positive correlation with root infection levels remains evident. Treatments maintaining optimal root infection (likely P2 or P3) hold strong potential for generating healthy, stable spore yields. However, at 42 days after planting (DAP), AMF prioritizes essential functions like nutrient transfer through arbuscules over sporulation, given the active host plant status and non-stressful environmental conditions (Oehl et al., 2009). Overall, these findings underscore the importance of integrated nutrient management when deploying AMF biofertilizers. Organic nutrient applications at optimal doses (e.g., P2–P3) deliver more balanced outcomes, preserving symbiotic benefits while supporting plant growth, in contrast to high-dose inorganic fertilizers (P1) that antagonize AMF colonization.

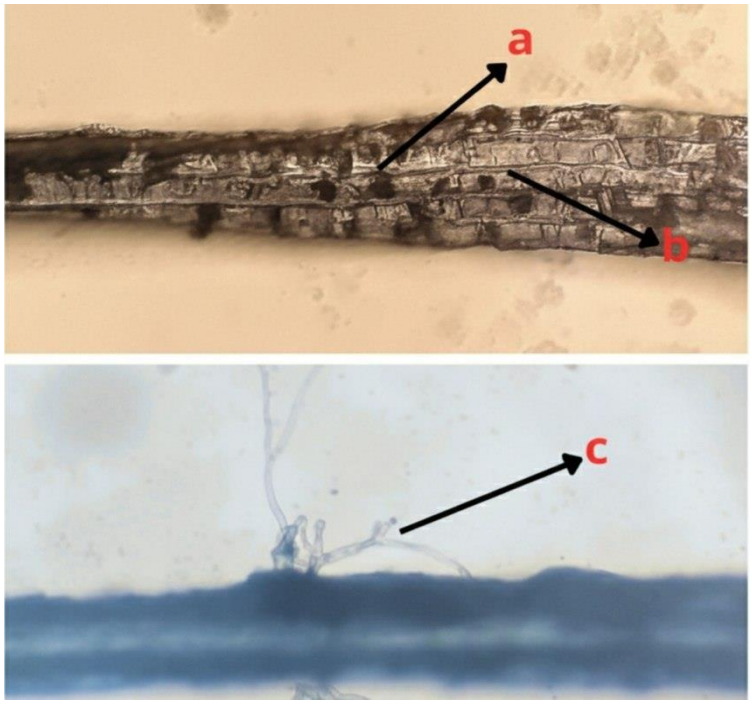

Figure 1. Microscopic verification of mycorrhizal presence and root infection intensity: a (vesicles), b (internal hyphae), and c (external hyphae).

Microscopic observation of sorghum roots infected with arbuscular mycorrhizal fungi (AMF) from Glomus sp. reveals a symbiotic association characterized by three principal structures: vesicles (a), internal hyphae (b), and external hyphae (c). AMF infection initiates as external hyphae (c)extensively distributed throughout the rhizosphere penetrate the host root epidermis. These extraradical structures are pivotal, functioning as root system extensions that substantially enhance acquisition of water and immobile soil nutrients like phosphorus (P), while also facilitating fungal reproduction and soil aggregation via glomalin secretion (Smith & Read, 2008; Rillig, 2000). Post-penetration, hyphae differentiate into internal hyphae (b) within root cortical cells, serving as conduits that channel externally absorbed nutrients to root cells through arbuscules (integral to internal hyphae, though unlabeled here) the primary nutrient exchange interfaces. AMF further develops vesicles (a), intracellular bubble-like structures in the root cortex that store lipids and nutrients as fungal food reserves, enabling persistence and serving as propagules for subsequent infections (Brundrett et al., 2008). The co-occurrence of these three structures confirms successful, fully functional AMF colonization, underpinning effective mutualistic nutrient exchange that bolsters sorghum nutrition and growth.

These findings carry significant implications for optimizing mass-scale AMF inoculum production. To maximize spore yields, growth media or host nutrient solutions (as employed in this hydroponic system) should be maintained at low nutrient levels particularly phosphorus or under controlled nutrient stress (Aryanto et al., 2018). Low-concentration cow urine POC (P2) supplies minimal essential nutrients sufficient to sustain sorghum host growth while imposing limitations that compel maximal AMF sporulation, positioning it as an efficient regimen for inoculum propagation.

Cow urine POC represents an organic fertilizer with notably higher phosphorus content compared to urine-derived fertilizers from other livestock, such as goats or rabbits. Its macronutrient profile includes organic C (1.460%), N (0.098%), P_2O_5 (0.102%), K_2O (0.216%), Ca (166.52 ppm), and Mg (104.61 ppm), alongside micronutrients comprising Co (2.15 ppm), Al (2.88 ppm), Fe (0.13 ppm), Na (1.28 ppm), Ni (0.21 ppm), Zn (0.23 ppm), B (1.13 ppm), and Mn (0.012 ppm). Additionally, it contains key hormones: IAA (8.61 ppm), cytokinin (5.16 ppm), and gibberellin (2.54 ppm) (Pratiwi et al., 2019). Application of cow urine POC to sorghum as an AMF host in inoculum production elicited varied growth responses.

Leaf Area

At 42 days after sowing, treatments P1, P3, and P4 produced the largest leaf areas (Table 1). The superiority of P1 (AB Mix 40 ppm) is logical, as this balanced inorganic nutrient solution delivers macro- and micronutrients in readily absorbable forms, optimizing photosynthesis rates and cell division. Instant nitrogen availability is particularly crucial for chlorophyll synthesis, which directly drives leaf area expansion the cornerstone of optimal plant height and leaf number. AB Mix thus serves as the gold standard for maximal vegetative growth. Optimal performance in P3 and P4 indicates that POC nutrient levels in these treatments effectively supported leaf expansion. Beyond nutrient supply, gibberellin content likely contributed, as these hormones promote stem elongation, cell enlargement, and synergistically with auxins and cytokinins vigorously enhanced biomass accumulation (Salisbury & Ross, 1995).

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Plant Height

Treatments P1, P3, and P4 yielded the greatest plant heights, consistent with their superior leaf area expansion (Table 1). Conversely, P0 and P2 showed no significant differences, grouping them in a lower-performance category. The resurgence of optimal growth in P3 and P4 underscores their effectiveness in promoting plant vigor. Plant height increases in this study were likely influenced by auxin (IAA), which drives cell elongation and apical dominance directly correlating with enhanced stature in P1, P3, and P4. Additionally, auxin stimulates root initiation and elongation, critical for arbuscular mycorrhizal fungi (AMF) inoculum production since AMF colonization targets the host root system.

Leaf Number

Statistical analysis from Table 1 necessitates rejection of the null hypothesis (H_0), indicating overall treatment differences. Descriptive observations reveal marked numerical variation, from the highest leaf count in AB Mix 40 ppm (P1: 7.6 leaves) to the lowest in cow urine POC 50 cc (P3: 5.6 leaves). However, at the 5% significance level, no statistically significant differences emerged among treatments. This implies that although AB Mix-treated plants averaged two more leaves than those receiving 50 cc POC, the numerical disparity lacked sufficient strength to attribute it definitively to fertilizer effects. Scientifically, such observed leaf count differences likely reflect substantial experimental error. Sources of this error include imperfectly controlled factors like minor genetic variation among seeds, micro-environmental differences across pots (e.g., temperature, drainage, aeration), or slight growing medium heterogeneity. High inter-replicate variability within treatments ultimately obscured any true fertilizer impacts.

In this study, leaf number was primarily governed by nutritional factors. High-nutrient treatments induced earlier leaf senescence and shedding, whereas low-nutrient regimes delayed this process. Rapid vegetative growth hinges on both nutrient availability and plant hormones; for leaf number specifically, cytokinins play a pivotal role by promoting cytokinesis (cell division), inhibiting senescence, and stimulating lateral bud and leaf development (Taiz & Zeiger, 2002). Effective cytokinin concentrations enhanced leaf number (JD42) and leaf area, aligning with the superior performance of P1 and P4 in these parameters. Expanded leaf area is crucial for photosynthetic efficiency, which fuels overall plant growth and sustains carbohydrate supply to the AMF symbiosis.

Fresh Weight

Study results (Table 1) indicate that nutrient-deprived plants (P0) yielded the lowest fresh weight at 3.39 g. Conversely, AB Mix instant nutrition (P1) excelled with 13.7 g, as its balanced, readily absorbable macro- and micronutrients optimized plant growth. Low POC doses (P2: 5.86 g; P3: 7.27 g; both group b) improved over the control but lacked statistical significance. P4 emerged as the top POC treatment at 12.86 g, rivaling P1's efficacy. This demonstrates cow urine POC's substantial potential at higher concentrations (75 cc/L) to match premier inorganic fertilizers for fresh biomass accumulation. P4 likely hit the "sweet spot" an optimal concentration approximating AB Mix's osmotic balance. Elevated nutrient levels enhance root osmotic pressure, equilibrating and maximizing water/nutrient uptake efficiency.

Significant fresh weight differences between AB Mix- and POC-treated plants arise from distinct cellular water and nutrient uptake mechanisms. AB Mix-treated roots rapidly absorb nitrate, accumulating it in vacuoles and spiking intracellular salt ion concentrations. This triggers intense osmosis, drawing substantial water into cells to equilibrate solute levels, rendering cells highly turgid and driving dramatic fresh weight gains (Taiz & Zeiger, 2010). In contrast, POC nutrients comprise complex molecules requiring gradual microbial or biological breakdown prior to absorption, precluding sudden salt ion surges. Consequently, POC plants avoid excessive water influx, yielding normally dense cellular structures without pronounced water-retention swelling.

Dry Weight

Table 1 reveals that P0, P2, and P3 exhibited the lowest dry weights, while P1 and P4 achieved the highest. The depressed dry weights in P0, P2, and P3 signify nutrient stress conditions. Dry weight serves as the most precise biomass accumulation metric, reflecting plant efficiency in converting solar energy and nutrients into organic tissues like carbohydrates, proteins, and cellular structures. Low dry weight signals severe metabolic disruption from nutrient deficiency. This primarily stems from impaired photosynthesis: macronutrient shortages disrupt chlorophyll synthesis, inducing chlorosis. Reduced chlorophyll directly curtails carbon assimilation rates, severely limiting photosynthate production (glucose, starch) the core biomass building blocks. Consequently, plants lack energy reserves for tissue mass expansion (Salisbury & Ross, 1995).

CONCLUSION

Cow urine POC concentrations significantly influenced arbuscular mycorrhizal fungi (AMF) spore inoculum production in sorghum host plants under hydroponic conditions. Lower concentrations (P0 and P2) generated the highest AMF spore yields. Cow urine POC concentrations also significantly affected key plant growth parameters plant height, leaf area, fresh weight, and dry weight while exerting no significant influence on leaf number. Higher nutrient concentrations optimized plant growth (P1 and P4) conversely, arbuscular mycorrhizal fungi (AMF) spore inoculum production declined.

The treatment of cow urine POC concentration on spor FMA inoculum production on sorghum host plants using a hydroponic system showed that low nutrient concentrations in treatments P0 and P2 produced maximum spore results, but plant growth did not show optimal outcomes. Future studies should identify optimal POC concentrations or nutrient solution modifications that balance AMF sporulation with host vigor. Additional investigation is warranted into mechanisms suppressing AMF sporulation at high POC levels (P1, P4). Research could involve analysis of specific compounds in cow urine POC and planting media at those concentrations that potentially suppress spore formation.

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