

RESEARCH ARTICLE



# Production technology and optimization of inputs for soil-less maize green fodder production

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#### **ARTICLE HISTORY**

Received: 29 November 2024 Accepted: 18 December 2024 Available online Version 1.0: 24 February 2025

() Check for updates

#### Additional information

**Peer review**: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc See https://horizonepublishing.com/journals/ index.php/PST/indexing\_abstracting

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#### **CITE THIS ARTICLE**

Thambiyannan S, Senthivelu M, Sathya Sheela K R V, Lakshmi Narayanan S, Satheesh Kumar N, Radhajayalakshmi R, Ramasamy K. Production technology and optimization of inputs for soil-less maize green fodder production. Plant Science Today (Early Access). https://doi.org/10.14719/pst.6406

#### Abstract

The study on seed priming with nutrients in maize was conducted to produce soil-less maize green fodder. The experiment was conducted in a completely randomized design with nine treatments replicated thrice. The treatments included Control (no priming), soaking seeds in water for 12 or 24 h, soaking seeds in urea (0.1% solution), Mono ammonium Phosphate (MAP) (0.1% solution) and in 19:19:19 (0.1% solution) for 12 or 24 h. To optimise the seed rate and harvesting time for soil-less fodder production, the experiment was laid out in a completely randomized design with four treatments and five replications. The seed rate of 400, 500, 600, and 700 g per square foot (sq. ft.) was adopted with harvesting time treatments at 7, 8, 9, and 10 days after germination. Results showed that a seed rate of 400 g per sq. ft. produced the highest germination rate, taller plants, and higher fodder yield and dry matter production (DMP), comparable to the 500 g per sq. ft. treatment. For seed priming, seeds soaked in a nutrient solution of 0.1% 19:19:19 for 24 h had the highest fodder yield followed by 24 h of soaking in 0.1% MAP and 24 h in 0.1% urea. Harvesting at 9 days after sowing (DAS) resulted in a higher fodder yield, DMP, and crude protein. These findings underscore the significant role of research in advancing the field of soil-less green fodder production. Based on the conducted experimental studies, density rate of 400 g per sq. ft. (equivalent to 4.31 kg m<sup>-2</sup>) is optimal for achieving higher yields of maize green fodder in soil-less production and seed priming with 19:19:19 nutrient solution at a concentration of 0.1% for 24 h has increased the green fodder yield by 75 % over control. Harvesting green fodder at 9 DAS is recommended under a low-cost hydroponic system.

#### **Keywords**

green fodder; maize; seed rate; seed priming; soil-less production; time of harvest

#### Introduction

In maize, a shortage of green fodder is a significant obstacle to enhancing livestock productivity (1). Less than 5 % of the total cultivated land is allocated for green fodder production (2). As of now, there is a growth in the population of cattle along with the increased demands for feed and fodder due to an intensive rearing system. Feeding fresh green fodder with high quality to dairy animals is an essential criterion to sustain productivity

economically (3). However, farmers face numerous significant constraints in the production of green fodder. These include small land holdings, less allocation or nonavailability of land for green fodder cultivation, irrigation water scarcity as well as quality of water, non-availability of fodder seeds with quality, natural calamities, etc. (4). The non-availability of quality fodder year-round exacerbates the challenges of sustainable dairy farming. The soilless production system is becoming an increasingly popular alternative to conventional fodder cultivation due to its ability to overcome these constraints and problems (5–7). Keeping animals has become quite common, even in urban areas where there is little space for growing green fodder. The high cost of production results from acquiring fresh green fodder and concentrated feed for the animals' daily needs.

The production of green fodder in open field conditions is fetched to low yield even with high water consumption due to climate change and adverse weather conditions. This situation paves the way for the emergence of soil-less production, a viable alternative for producing green fodder for daily requirements. The soil-less production, the art and technology of soil-less plant cultivation, is revolutionizing green fodder production in the 21st century (7). This is the concept of soil-less production, growing crops without soil using water and nutrients. This process occurs in a hydroponic system using only water and nutrients to produce an abundant and nutritious grass and root combination (8, 9).

The soil-less production may be done in fully automated hydroponics fodder production systems, where the sensors will control the water, light, temperature, humidity, and aeration (10), which may be high-cost with a hightech or low-cost with a structure developed by using the locally available materials considering the principle of the seed germination. Maize is an important cereal with various uses. In addition to being consumed as grain, it is also utilized as animal fodder due to its high fresh biomass. Maize is a better choice for soil-less production as fodder due to its availability, higher biomass, higher seed-tobiomass ratio, and faster growth (11). The technology of soil-less fodder production is a new development in India, and all the research focuses on scientifically standardising agro-techniques for fully automated hydroponics fodder production systems (Controlled conditions). Soil-less agriculture takes care of plant growth from soil-related issues like salinity, non-arable soil, soil-borne pests and diseases, and poor soil quality. The last several decades have seen a surge in interest in the commercial use of soil-less farming, which has sparked a lot of research into new growing systems and a deeper comprehension of crop physiology and how it affects quality factors (12).

Investment in high-cost hydroponics production units is quite difficult for medium and small farmers. In addition, having high-cost production units by animal rearers in urban areas may be very difficult. In addition, the technologies for low-cost soil-less green fodder production have not been explored as much as automated systems. Hence, it is essential to explore the possibilities of low-cost soil-less production systems, and the technologies for the low-cost soil-less production system are highly essential at this juncture. This research focused on developing agro technologies for low-cost structure development by using locally available materials, *viz.*, optimizing seeding density, priming seeds with nutrients, and harvesting period, which are the main factors in agrotechniques that influence productivity, profitability, and quality.

#### **Materials and Methods**

The soil-less maize green fodder production experiments were conducted in 1 cent area under a shade net at Maize Research Station, Vagarai, Tamil Nadu, India to find out the optimum seed rate, the impact of seed priming with or without chemicals, and to find out the optimum time of harvest for higher green fodder yield under the low-cost the soil-less production system. The research station is located at an altitude of 254 m above sea level at latitude 10°57' N and longitude 77°56' E. It is in the Western Agro-Climatic Zone of Tamil Nadu state, India.

#### Establishment of a low-cost soil-less production system

The shade net structure was established by using wooden reapers. Two rack assemblies were erected with wooden reapers and arranged perpendicular to the brick wall. The racks were erected with a length of 2 m and a width of 0.6 m. A height distance of 40 cm between the two racks was kept for easy handling. The entire rack system was covered with a 90 % shade net, and one side door was provided to enter the soil-less production system (Pic. 1).



Pic. 1. Trays arranged in the shade net structure.

To cultivate the soil-less green fodder production, trays measuring 60 cm in length by 40 cm in width and 5 cm in depth, with small holes at the bottom for draining excess water, were utilized. The trays were made of polypropylene and were strong enough to hold the weight of the green fodder (Pic. 2).

#### **Experiment details**

The experiment on seed rate was conducted with four treatments in a completely randomized design (CRD) and was replicated six times. The seeding density treatments were fixed per square foot for easy recommendations to the farmers. The treatments were 400 g per square foot (sq. ft.) (4.31



Pic. 2. Production of maize green fodder under a soil-less production system. (A) Soaking of grains; (B) Incubating by covering with cloth; (C) Grains in the soil-less production trays; (D)Sprouting of grains; (E) Initiating of shoots; (F) Initiating of shoots; (G) Initiating of shoots and roots; (H) Establishment of green fodder; (I) Root development in hydroponic and (J) Fully established fodder.

kg m<sup>-2</sup>), 500 g per sq. ft. (5.38 kg m<sup>-2</sup>), 600 g per sq. ft. (6.46 kg m<sup>-2</sup>) and 700 g per sq. ft. (7.53 kg m<sup>-2</sup>). The experiment on seed priming with nutrients was conducted using nine treatments in a CRD and replicated thrice. The treatments were T<sub>1</sub> - Control (No priming), T<sub>2</sub> - 12 h water soaking, T<sub>3</sub> -24 h water soaking, T<sub>4</sub> - 12 h soaking in urea (0.1% solution),  $T_5$  - 12 h soaking in MAP (0.1% solution),  $T_6$  - 12 h soaking in 19:19:19 (0.1% solution), T<sub>7</sub> - 24 h soaking in urea (0.1% solution), T<sub>8</sub> - 24 h soaking in MAP (0.1% solution) and T<sub>9</sub> - 24 h soaking in 19:19:19 (0.1% solution). The experiment on the harvesting time was conducted with four treatments in CRD and replicated six times. The treatments were harvested on the seventh day, the eighth day, the ninth day, and the tenth day after germination. All three experiments were repeated once again as confirmatory experiments.

#### **Experiment procedures**

Regarding the seeding density and time of harvest experiments, grains were soaked in water for 24 h to improve their ability to absorb water quickly. This helps with the metabolism and use of reserved food for growth and development. In the experiment on seed priming, seeds were soaked in water or nutrient solutions for 12 h or 24 h as per the treatments. Then, water was drained out, and the maize grains were incubated by covering them with cloth and kept for 24 h to initiate the process of germination. The sprouted grains were spread in the tray and watered every 2 h during the daytime using a handheld rose-can at the rate of 1L per tray every day.

#### **Experiment observations and analysis**

The germination percentage was recorded on the 3rd day after the transfer to the trays. The green fodder was harvested after 9 DAS in the seed rate and seed priming experiments and as per the treatments at the time of the harvest experiment. Green fodder with roots was weighed and expressed in kilograms (kg). The fodder mat was torn apart and mixed to obtain a representative sample for quality analysis. Representative fresh samples were weighed and then allowed to air dry in a well-ventilated area. Again, the samples were dried in an oven at 70°C until a constant weight was noticed. Then, they were milled in a Wiley mill with a 1 mm sieve size before undergoing chemical analysis. Dry matter and nitrogen (N) were determined, and N × 6.25 was used to calculate crude protein (CP). Raw data of all the replications were processed to calculate the means and standard deviations. All data used for one-way analysis of variance (ANOVA) passed the normality test with a five per cent (0.05) probability, and the results were interpreted (13).

#### **Results and Discussion**

#### **Optimizing Seeding Density**

The seeding density significantly influenced maize grain germination in the low-cost soil-less production system (Table 1). The lowest seed rate recorded a higher seed germination percentage of 79.2 (+2.2) (Expt. 1) and 79.5 (+1.7) (Expt. 2); whereas the increase in seeding density resulted in decreased germination percentage. The seeding density from 400 to 600 g per sq. ft. was comparable; it significantly reduced germination beyond this point. The lowest germination percentages were 73.8 and 74.1 in Expt. 1 and Expt. 2, respectively, registered under the 700 g per sq. ft. seeding density. The decreasing germination percentage while increasing grain density may be due to the overlapping of grains and poor availability of light and space. A seeding density of 400 g per sq. ft. resulted in the tallest plants, measuring an average of 23.3 cm (± 2.1) and 23.7 cm (± 1.8) in Expt. 1 and Expt. 2, respectively. This was followed by a seeding density of 500 g per sq. ft., which produced comparable heights. In contrast, a seeding density of 700 g per sq. ft. led to shorter plants, averaging 19.4 cm (± 1.6) and 20 cm (± 1.2) in Expt. 1 and Expt. 2, respectively. When seeding density exceeds the optimal level, it negatively impacts the growth of maize seedlings in hydroponic trays (14), and excessive density leads to inefficient growth and increased water and energy usage. Higher seeding density creates competition among the seedlings, adversely affecting germination rates and plant height, as reported in earlier studies (15). Regarding crude protein, the crude protein content decreased slightly as the seed rate increased, but the difference was not significant.

The green fodder (fresh) yield per unit area (m<sup>2</sup>) of the soil-less cultivated maize was statistically significant for different seeding densities (Fig. 1). Yield is the result of various metabolic processes occurring at various stages of a plant's growth. Among the seed density, higher green fodder production per unit area (31.4 kg m<sup>-2</sup>, 29.8 kg m<sup>-2</sup> in Expt. 1 and Expt. 2, respectively) was recorded in the treatment having higher density (700 g per sq. ft. (7.53 kg  $m^{-2}$ )), and it was comparable up to the reduced density of 500 g per sq. ft. (5.38 kg m<sup>-2</sup>). The lower seed density of 400 g per sq. ft. recorded significantly lower maize green fodder yield per unit area (6.2, 5.9 kg kg<sup>-1</sup> of grains in Expt. 1 and Expt. 2, respectively). Higher green matter yield per m<sup>2</sup> was higher under higher seed density due to higher seed rate, and the same trend is firmly observed in the findings (16). Among the different seeding densities, grains sown @ 400 g per sq. ft. recorded higher green fodder yield (26.9 and 25.6 kg m<sup>-2</sup> in Expt. 1 and Expt. 2, respectively) followed by 500

Table 1. Influence of seeding density on growth, yield, and crude protein content of the soil-less maize green fodder production.

Treatments	Germination (%)		Plant he	ight (cm)	Crude protein (%)		
	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2	
T <sub>1</sub> – 400 g per sq.ft. (4.31 kg m <sup>-2</sup> )	79.0 <u>+</u> 2.2 <sup>a</sup>	79.5 <u>+</u> 1.7 <sup>a</sup>	23.3 <u>+</u> 2.1 <sup>a</sup>	23.7 <u>+</u> 1.8 <sup>a</sup>	10.41 <u>+</u> 0.50 <sup>a</sup>	10.39 <u>+</u> 0.47 <sup>a</sup>	
T <sub>2</sub> – 500 g per sq.ft. (5.38 kg m <sup>-2</sup> )	78.6 <u>+</u> 2.5ª	78.7 <u>+</u> 1.78ª	22.2 <u>+</u> 1.7 <sup>ab</sup>	22.7 <u>+</u> 1.1 <sup>ab</sup>	10.80 <u>+</u> 0.39ª	10.76 <u>+</u> 0.36 <sup>a</sup>	
T <sub>3</sub> – 600 g per sq.ft. (6.46 kg m <sup>-2</sup> )	77.6 <u>+</u> 2.3ª	77.7 <u>+</u> 2.0ª	20.7 <u>+</u> 1.1 <sup>bc</sup>	21.3 <u>+</u> 1.3 <sup>bc</sup>	10.31 <u>+</u> 0.38ª	10.32 <u>+</u> 0.38 <sup>a</sup>	
T <sub>4</sub> – 700 g per sq.ft. (7.53 kg m <sup>-2</sup> )	73.8 <u>+</u> 2.2 <sup>b</sup>	74.1 <u>+</u> 2.1 <sup>b</sup>	19.4 <u>+</u> 1.6 <sup>c</sup>	20.0 <u>+</u> 1.2 <sup>c</sup>	10.22 <u>+</u> 0.34 <sup>a</sup>	10.24 <u>+</u> 0.42 <sup>a</sup>	

Means with common superscript letters in a column are not significant at P < 0.05.



Fig. 1. Influence of seeding density on maize green fodder yield.

g per sq. ft. and 600 g per sq. ft. The 700 g per sq. ft. seed rate recorded lesser green fodder yield (4.07 kg kg<sup>-1</sup> of grains). A similar trend of results was also evident with DMP (Fig. 2). Higher yield parameters, including the fresh weight of the shoot, root and the total dry matter accumulation in these parts, contribute to the increased yield of maize green fodder. It has also been reported that there is a specific augmented yield of fresh green fodder from maize per kilogram of seeds when using an optimum seed rate (16–18).



**Fig. 2.** Influence of seeding density on maize green fodder DMP and crude protein content.

A significant decline in biomass yield per kilogram of seeds was observed when seeding density exceeded 400 g per sq. ft. This decrease in biomass production per kilogram of maize grains may be attributed to competition among the maize sprouts in a soil-less production system. Additionally, it has been reported that increasing maize seeding density in soil-less cultivation leads to greater microbial contamination in the green fodder, particularly in the root area, which negatively affects both the growth and quality of the fodder (5). Green fodder production under the existing system (soil-based) needs more area, is labourious, and is time- and input-consuming. Through this low-cost soil-less production system, we can eliminate all the issues faced in the existing system.

The cost of producing green fodder per kg increased with increasing seeding densities, and gross income also increased with increasing seeding densities (Fig. 3). However, the return per rupee invested was better at the seeding density of 400 g per sq. ft. (4.31 kg m<sup>-2</sup>). While increasing the seeding density, the return per rupee invested decreased. The above results show that increasing the seeding density per unit area will increase the yield per unit area. On the other hand, the yield per kg of seeds will decrease while increasing the seeding density. However, the increase in green fodder yield per unit area may not be productive due to the increase in seed rate per unit area. Therefore, a seeding density of 400 g per sq. ft. (4.31 kg m<sup>-2</sup>) will yield quality green fodder with higher profits.



 $T_1 - 400$  g per sq. ft.,  $T_2 - 500$  g per sq. ft.,  $T_3 - 600$  g per sq. ft. and  $T_4 - 700$  g per sq. ft. BCR: Benefit-cost ratio - Cost of production per kg of green fodder (Rs.)

## Influence of seed priming on the soil-less production of fodder

Maize grain priming significantly influences germination as well as plant growth of maize in soil-less production; all the treatments with 24 h of soaking recorded higher germination and taller plants (Table 2). Germination and emer-

Table 2. Influence of maize grain priming on growth, yield, and crude protein content of fodder under low-cost soil-less production system.

Treatment	Germination (%)		Plant height (cm)		Fodder yield (kg kg <sup>-1</sup> grains)		DMP (g kg <sup>-1</sup> grains)	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2
T <sub>1</sub> – Control (No priming)	50.4	54.2	12.6	14.0	2.54	2.59	294	300
T <sub>2</sub> – 12 h soaking in water	65.0	71.0	17.9	19.0	3.40	3.72	402	428
T <sub>3</sub> – 24 h soaking in water	78.1	77.9	19.7	21.9	3.76	4.04	439	464
T <sub>4</sub> – 12 h soaking in urea	70.5	73.5	20.1	22.4	3.71	4.01	497	529
T <sub>5</sub> – 12 h soaking in MAP	71.4	73.5	20.2	23.1	3.84	4.07	513	537
T <sub>6</sub> – 12 h soaking in All-19-nutrient solution	68.0	73.0	20.6	23.3	3.94	4.08	533	538
T <sub>7</sub> – 24 h soaking in urea	80.4	78.6	21.7	25.0	4.44	4.23	647	604
T <sub>8</sub> – 24 h soaking in MAP	79.4	78.2	22.4	26.1	4.70	4.41	674	630
$T_{9}$ – 24 h soaking in All-19-nutrient solution	81.7	79.8	23.1	26.1	4.81	4.55	706	650
SED	2.6	2.4	0.9	0.7	0.2	0.17	23	20
CD (P < 0.05)	5.3	4.8	1.9	1.5	0.5	0.344	46	41

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gence of maize grains are essential to achieve higher green fodder yield per unit area in a short span of time. Seeds primed in the nutrient solution of 0.1 % of 19:19:19 (All-19nutrient solution) for 24 h recorded higher germination percentage (81.7 and 79.8 % in Expt. 1 and Expt. 2, respectively) and followed by 24 h soaking in 0.1% urea, 0.1 % MAP, and in water, and all these treatments were comparable. The lowest germination percentage (50.4 and 54.2 % in Expt. 1 and Expt. 2, respectively) was recorded under control (no soaking), and the grains soaked in water for 12 h registered a better germination percentage than the control. The grains soaked in water for 24 h also significantly performed better germination than grains soaked in water for 12 h. Variables that affect the effects of maize grain priming include the priming solutions and the timing of the priming (19). It clearly supported that seed priming activates several biochemical processes in the seed, including hydrolysis, breaking of dormancy, enzyme activation, and improving seed germination (20). Also, using micronutrients in priming resulted in higher seed germination percentage (21) and better performance under longertime seed priming (22).

Maize grains priming with nutrients had a significant impact on the growth of the plants. Grains primed with 24 h registered higher plant height except grains soaked in water alone. The same trend was also registered in the treatments where grains were soaked for 12 h. Seeds primed with nutrient solution improved the establishment of seedlings compared to those primed with water alone and non-primed seeds. Similar conclusions were drawn (23) in support of these findings: priming with solution improves the thickness, height and weight of maize seedlings. Earlier uptake of nutrient solutions that may have activated the germination process may have contributed to improvements in the growth and development of maize seedlings. Poor growth of maize green fodder was observed in the control treatment in which priming was not practiced.

Seeds primed in nutrient solution of 0.1 % of All-19nutrient solution 9 for 24 h recorded higher fodder yield, followed by 24 h soaking in 0.1 % MAP and 24 h soaking in 0.1 % urea, and all these treatments were comparable. The most negligible fodder yield was recorded under control (no soaking). Maize grain is primed with nutrient solution to increase green fodder yield, which is mainly due to the combined application of nitrogen, phosphorus, and potassium. Moderate fertilization with nitrogen, phosphorus, and potassium can effectively enhance the nutrient content of plants, thus improving crop yield and quality. The cumulative effect on the maize green fodder is mainly due to nitrogen application, which plays a key role in meristematic activity and cell division. Improved vegetative growth and dry matter accumulation may result from increased cell number. Potassium is crucial in activating enzymes responsible for protein synthesis and carbohydrate translocation. This can lead to stronger root development, increased plant growth, and higher fodder yield. Applying nutrients significantly increased the white and yellow maize yield compared to the control (24). A similar result Maize can produce more green fodder when its seeds are primed with macronutrients, resulting in a significant increase in fresh weight for the shoot and root. This is due to the accumulation of dry matter at various growth stages, which is then distributed across different crop parts, such as the shoot and root. The increased accumulation of dry matter and its movement to different parts of the crop is likely due to higher photosynthetic activity, which is influenced by a nutrient supply that is synchronized with the crop's demand.

The crude protein content of maize green fodder was not altered significantly due to priming with nutrient solutions. However, seeds primed in 0.1 % All-19-nutrient solution for 24 h recorded numerically higher crude protein content, followed by 0.1 % MAP and 0.1 % urea and 24 h of soaking of maize grain in water and 12 h in nutrient solutions. The least crude protein content was recorded under control (no soaking).

#### **Optimizing the time of harvest**

Maize green fodder was harvested at four different intervals, the growth, yield and nutritional content were presented in Fig. 4. The plant height increased significantly up to 9 days and on the 10<sup>th</sup> day the increase in plant height was insignificant. Plants harvested at 10 days after sowing (DAS) were taller (24.76 cm) than those harvested on the 9<sup>th</sup> day (23.23 cm), and their heights were comparable. The lowest plant height was recorded when harvested on 7<sup>th</sup> day (17.02 cm). In this study, we observed that plant growth became insignificant after the ninth day, and then began to weaken. The same results were obtained in the soil-less-grown maize (25).



Fig. 4. Influence of time of harvest on growth, yield, DMP and crude protein.  $T_1 - 7$  DAS,  $T_2 - 8$  DAS,  $T_3 - 9$  DAS and  $T_4 - 10$  DAS.

Green fodder production is highly correlated to harvest time with a correlation coefficient  $R^2$  =0.95. Harvesting at the 10 DAS recorded higher fodder yield (4.88 kg kg<sup>-1</sup> grains), followed by harvesting on the 9<sup>th</sup> day (4.63 kg kg<sup>-1</sup> grains), and both harvest time were comparable. The lowest fodder yield was registered under harvesting on the 7th day (3.02 kg kg<sup>-1</sup> grains). A hydroponic system's growth and yield mainly depend on the resources available in the

seeds; until the resources are available, the growth and biomass tend to increase; after exhausted resources, the growth and biomass decrease. A similar trend was also observed in sorghum fodder production in hydroponics (26). If harvesting is delayed, it will increase biomass production under the soil-less production system but decrease dry matter and dry fodder yield (27).

The harvesting stage significantly influenced DMP of hydroponically grown fodder maize. As the harvesting stage was delayed, DMP increased significantly up to 9 days. After the 9<sup>th</sup> day, the increase was not significant. This might be due to the reduction of dry matter content by diminishing starch substances utilized for plant development, as reported in the hydroponically grown barley (28).

In addition to being an essential nutrient for ruminant animals, crude protein is a primary quality parameter of fodder (29). Crude protein content increased as the harvest time was delayed up to 9 days and then decreased. Fodder harvested on the 9<sup>th</sup> day recorded higher crude protein content, followed by harvesting on the 10th day, and both were comparable. The least crude protein content was recorded under harvesting on the 7<sup>th</sup> day. An earlier study also concluded, similar to our findings, that the maize green fodder crude protein content increased following delayed harvest days (11). However, for effective utilization of low-cost soil-less production systems, it has to be sanitized regularly and provide some time gap between the two production cycles to avoid microbial contamination.

#### Conclusion

Based on the conducted experiments, it has been determined that a density rate of 400 g per sq. ft. (equivalent to 4.31 kg m<sup>-2</sup>) is optimal for achieving higher yields of maize green fodder in soil-less productions. To increase the yield further, it is recommended to prime the grains with an All-19-nutrient solution at a concentration of 0.1% for 24 h. Green fodder should be harvested for the best quality under a low-cost soil-less production system at the 9 DAS. This low-cost soil-less production system may provide the solution for green fodder supplements during lean seasons. In addition, additional variables, such as light intensity or temperature control, should be explored to optimize soil-less fodder production further.

#### Acknowledgements

The authors are grateful to Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India, and acknowledge the work of the Late Professor (Agronomy) Dr. M. Mohamed Amanullah for his conceptualization, methodology, investigation, and formal analysis.

#### **Authors' contributions**

ST and SM contributed to conceptualization and methodology. ST and SM conducted the investigation. ST, SM, SKRV, and LS were involved in data curation, resources, and validation. ST and SM wrote the original draft. ST, SKRV, and LS performed formal analysis. ST, SM, SKRV, LS, SN, RR, and KR contributed to writing - review and editing. All authors read and approved the final manuscript.

#### **Compliance with ethical standards**

**Conflict of interest**: The authors declare no conflict of interest.

Ethical issues: None

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