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Impact of Cow Urine and Substrate Composition on Germination and Early Growth of Papaya (*Carica papaya* L.) Seedlings

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Authors' contributions

This work was carried out in collaboration among all authors. Authors RKY and PS conceptualized the research and designed the experiments. Author PS executed the field/lab experiments and data collection. Authors PS, RKY, MCJ and MCB managed analysis of data and interpretation. Authors RKY and PS prepared the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study evaluates the impact of cow urine and substrate composition on the germination and early growth of papaya (*Carica papaya* L.) seedlings, a critical phase for successful crop establishment.
Study Design and Methodology: Conducted as a factorial experiment, it assessed eleven substrate or growing media combinations with and without cow urine treatment.

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Results: Results reveal that cow urine significantly reduces the time to 90 percent germination (T90) and enhances the germination index, indicating its stimulatory effect on seed metabolic activity. Treatments combining cow urine with substrates like soil, sand, vermiculite, cocopeat, and perlite (T_{10}) showed the shortest T90 and highest germination index, suggesting cow urine accelerates germination. Additionally, cow urine application improved growth metrics such as relative growth rate (RGR) and total biomass accumulation, with T_{10} and T_8 treatments yielding the highest values. Enhanced leaf relative water content (LRWC) and elevated proline levels in cow urine-treated plants indicate better water retention and stress resilience. Strong positive correlations were observed among proline content, RGR, and biomass accumulation, highlighting cow urine's contribution to vigorous seedling growth and stress adaptation.

Conclusion: The findings suggest that cow urine, in combination with optimized substrates, is a sustainable growth enhancer that promotes robust seedling development, suitable for organic farming practices aimed at papaya cultivation.

Keywords: Cow urine; substrate composition; papaya; germination index; proline; sustainable agriculture.

1. INTRODUCTION

The papaya (*Carica papaya* L.) is a tropical fruit belonging to the family Caricaceae, known for its high nutritive and medicinal value (Koul et al., 2022). It is native to tropical America and was introduced to India in the 16^{th} century. In India, it is extensively grown in various states such as Andhra Pradesh, Gujarat, Karnataka, Madhya Pradesh, Maharashtra, West Bengal, Chhattisgarh, Tamil Nadu, Assam, and Kerala (Anonymous, 2023). It is a rich source of Vitamin A (2020 IU/100g) and Vitamin C (85 mg/100g) and also provides fiber, magnesium, copper, folate, and pantothenic acid (Meena et al., 2017; Nkurunziza et al., 2022). Papaya fruit is very low in calories (43 calories/100g) and contains no cholesterol. It has 89.6 percent moisture, 9.5 percent carbohydrate, 0.5 percent protein, 0.1 percent fat, 4.0 percent calorific value, 0.4 percent minerals, 0.01 percent calcium, 0.01 percent phosphorus, 0.4 mg iron, 40 IU thiamine (Vit-B1), and 250 IU riboflavin (Vit-B2) (Bishnoi et al., 2024). It is one of the fruits that has gained popularity due to its quick returns, easy cultivation, and above all, its attractive and delicious taste; the wholesome fruit has multifarious uses.

Papaya is commercially propagated by seed and takes 3-5 weeks to germinate (Desai et al., 2017). Seed germination is affected by many factors, including the type of substrate used and environmental factors such as oxygen, water, temperature, and light for some plant species (Corbineau & Come, 2017). Seed treatment is essential to promote germination and reduce germination time with suitable growing media. Cow urine is a unique dairy by-product with properties such as manure, antimicrobial agent, and disinfectant. It contains 95 percent water, 2.5 percent urea, and 2.5 percent enzymes (Randhawa & Sharma, 2015; Kushwaha et al., 2024). In organic farming, cow urine is used in the preparation of various growth promoters and bio-pesticides, which effectively improve soil fertility and manage pests and diseases across diverse groups.

Growing media or substrates are defined as all those materials, other than soil, which alone or in mixtures can provide better conditions than agricultural soil (in one or more aspects). These media, from various origins, serve as substitutes for soil, providing anchorage for the root system, supplying water and nutrients to the plant, and ensuring adequate aeration in the root zone (Gruda et al., 2006; Balliu et al., 2021). The composition of the medium influences seedling quality (Wilson et al., 2001). Many grades of sand are available and can be used as a growing medium or as a component of various substrate mixtures to improve drainage properties. Pure sand is commonly used in deserts and coastal plains, as it is an inexpensive, local, natural resource (Shahid et al., 2013). Perlite and vermiculite have long been used to amend professional potting soils made from peat moss (known as 'soilless' mixes or artificial soils because they contain no soil) (Meena et al., 2017). Cocopeat, an agricultural by-product obtained after extracting fiber from coconut husks, is valued as a growing medium component with acceptable pH, EC, and other chemical attributes (Atzori et al., 2021). In view of the above facts, the present investigation was undertaken to study the effect of different growing media and cow urine on seed germination, growth and quality of papaya seedlings.

2. MATERIALS AND METHODS

2.1 Study Area and Treatment Application

A pot experiment on papaya (*Carica papaya* L.) cultivar Arka Surya was conducted in July 2019 at the nursery unit of the Department of Horticulture, Agricultural University of Kota, Rajasthan, located in a semi-arid climatic region. The experiment followed a factorial completely randomized design with three replications. This experiment consisted of two factors: the first factor was the growing or rooting media, and the second factor was cow urine. Eleven media combinations, with or without cow urine were used and resulting in a total of 22 treatments viz., soil (control) (T_0), soil + sand (1:1) (T_1), soil + vermiculite (1:1) (T_2), soil + cocopeat (1:1) (T_3), soil + perlite (1:1) (T_4), soil + vermiculite + perlite (1:1:1) (T_5), soil + vermiculite + cocopeat (1:1:1) (T_6), vermiculite + perlite + cocopeat (1:1:1) (T_7), soil + sand + vermiculite + perlite (1:1:1:1) (T_8), soil + sand + vermiculite + cocopeat (1:1:1:1) (T_9), and soil + sand + vermiculite + cocopeat + perlite (1:1:1:1:1) (T_{10}). To prepare the cow urine treatment, a small amount of cow urine was first diluted with water to make a 15% solution, which was then further mixed with 1 liter of water to achieve the desired concentration. Papaya seeds were soaked in this cow urine solution for 24 hours before being sown in trays.

2.2 Evaluation of Seedling Parameters

The time to 90 percent germination (T90) was calculated based on the number of days taken to reach 90% germination. Whereas, the germination index was calculated as described in the AOSA (1983) using the following formula:

$$\text{Germination index} = \frac{\text{No. of germinating seeds}}{\text{Days of first count}} + \frac{\text{No. of germinating seeds}}{\text{Days of final count}}$$

The Relative Growth Rate (RGR) calculated with the formula given by Fisher (1921) as follows:

$$\text{RGR (g/ g/ day)} = \frac{\text{In(W2)} - \text{In(W1)}}{\text{T2} - \text{T1}}$$

Where: W_1 = Initial dry weight of the plant (g) at time T_1. W_2 = Final dry weight of the plant (g) at time T_2. T_2 - T_1 = Time interval (days) between the two successive measurements.

Total biomass accumulation (g) is measured by harvesting the entire plant and weighing its dry weight after drying the plant material in an oven. However, the relative water content in recently matured leaves was determined, following the method suggested by Barrs & Weatherley (1962). The relative water content was estimated using the following formula:

$$\text{RWC (\%)} = \frac{\text{Fresh weight} - \text{Oven dry weight}}{\text{Turgid weight} - \text{Oven dry weight}} \times 100$$

Proline content was estimated using a rapid colorimetric method as suggested by Bates et al. (1973) and expressed as μM g^{-1} FW of leaf tissue.

2.3 Data Analysis

The data pertaining to various attributes were subjected to statistical analysis following Factorial CRD as suggested by Panse & Sukhatme (1985). Correlation (Person) among the various parameters was analysed using R software (R version 4.2.3, India).

3. RESULTS AND DISCUSSION

3.1 Seed Germination Attributes

The results of the study revealed significant variations in the time to 90 percent germination (T90) and germination index of papaya as influenced by different growing media and cow urine application (Table 1). For time to 90 percent germination (T90), the treatments showed a range of significant responses. In the absence of cow urine, T_{10} -Soil + Sand + Vermiculite + Cocopeat + Perlite (1:1:1:1:1) exhibited the shortest T90 (14.50 days), while T_0 required the longest (17.00 days). With the application of cow urine, T_{10} -Soil + Sand + Vermiculite + Cocopeat + Perlite (1:1:1:1:1) maintained the lowest T90 at 14.10 days, indicating a consistently faster germination response. The overall mean T90 across all treatments was reduced from 15.10 days without cow urine to 14.66 days with cow urine, highlighting cow urine's role in expediting the germination process. In terms of the germination index, treatments varied significantly during the experimentation. The germination index was higher with cow urine and in T_{10} -Soil + Sand + Vermiculite + Cocopeat + Perlite (1:1:1:1:1) achieving the highest value (6.00), followed by T_9 -Soil + Sand + Vermiculite + Cocopeat (1:1:1:1) (5.87) and T_8 -Soil +

Sand + Vermiculite + Perlite (1:1:1:1) (5.63). Without cow urine, T_8 exhibited the highest germination index (5.28), indicating that both the type of media and cow urine application enhanced seed vigor. Across all treatments, the germination index mean increased from 4.30 without cow urine to 4.91 with cow urine.

The significant reduction in time to 90 percent germination (T90) and increased germination index with cow urine application across treatments highlights the stimulatory role of cow urine on seed metabolic activity. The organic constituents in cow urine likely accelerated enzymatic activity and energy mobilization within the seed, expediting radicle emergence and overall germination speed (Thakur et al., 2023). The enhanced germination index with treatments involving cow urine, particularly in media compositions rich in components like vermiculite, cocopeat, and perlite, suggests that these substrates not only provide an optimal physical environment but, when combined with cow urine, create favorable biochemical conditions for seedling vigor. This effect can be attributed to the improved oxygen availability, moisture retention, and nutrient release dynamics in the media, supporting rapid seedling development. Similar results were observed by Shafique et al. (2021) in marigold, Verma et al. (2022) in Indian barberry and Desai et al. (2017) and Sharma et al. (2024) in papaya.

3.2 Seedling Growth Attributes

The findings on the Relative Growth Rate (RGR) and total biomass accumulation of papaya under different treatments indicate a significant enhancement in growth metrics with the application of cow urine across various growing media (Table 2). For Relative Growth Rate (RGR), values ranged from 2.56 g/g/day in T_0 without cow urine to a peak of 5.80 g/g/day in T_{10} -Soil + Sand + Vermiculite + Cocopeat + Perlite (1:1:1:1:1) with cow urine. The mean RGR across all treatments increased from 4.30 g/g/day without cow urine to 4.60 g/g/day with cow urine, suggesting that cow urine consistently boosted growth rate. While, treatments T_{10} -Soil + Sand + Vermiculite + Cocopeat + Perlite (1:1:1:1:1) and T_8 -Soil + Sand + Vermiculite + Perlite (1:1:1:1) showed the highest RGR values (5.56 and 5.28 without cow urine; 5.80 and 5.33 with cow urine, respectively), highlighting the synergistic effect of specific media types combined with cow urine on RGR. The data related to Total Biomass Accumulation revealed that treatments with cow urine application consistently led to higher biomass accumulation. The highest biomass was recorded in T_{10} -Soil + Sand + Vermiculite + Cocopeat + Perlite (1:1:1:1:1) (0.40 g without cow urine and 0.42 g with cow urine), registered a mean of 0.41 g. Across all treatments, the average total biomass accumulation increased from 0.29 g without cow urine to 0.31 g with cow urine.

Table 1. Effect of growing media and cow urine on time to 90 percent germination (T90) and germination index of papaya

Treatments	Time to 90 percent germination (Days)			Germination index		
	Without cow urine	**With cow urine**	**Mean**	**Without cow urine**	**With cow urine**	**Mean**
T_0	17.00	15.70	16.35	2.56	3.77	3.16
T_1	15.50	14.73	15.12	3.55	3.70	3.63
T_2	15.80	14.87	15.33	3.43	4.37	3.90
T_3	14.80	14.20	14.50	4.15	4.33	4.24
T_4	15.30	14.60	14.95	4.23	5.00	4.62
T_5	14.60	15.20	14.90	4.98	5.23	5.11
T_6	15.00	14.50	14.75	4.67	5.27	4.97
T_7	14.60	14.70	14.65	4.03	4.83	4.43
T_8	14.60	14.20	14.40	5.28	5.63	5.46
T_9	14.40	14.50	14.45	4.89	5.87	5.38
T_{10}	14.50	14.10	14.30	5.56	6.00	5.78
Mean	15.10	14.66		4.30	4.91	
Factors	**SE(m) ±**	**C.D. (5%)**		**SE(m) ±**	**C.D. (5%)**	
Media (M)	0.17	0.49		0.05	0.14	
Cow urine (C)	0.07	0.21		0.02	0.06	
M × C	0.24	0.69		0.07	0.19	

Table 2. Effect of growing media and cow urine on relative growth rate and total biomass accumulation of papaya

Treatments	Relative growth rate (g/ g/ day)			Total biomass accumulation (g)		
	Without cow urine	With cow urine	Mean	Without cow urine	With cow urine	Mean
T_0	2.56	3.26	2.91	0.20	0.25	0.23
T_1	3.55	3.75	3.65	0.26	0.28	0.27
T_2	3.43	3.87	3.65	0.25	0.27	0.26
T_3	4.15	4.43	4.29	0.28	0.29	0.29
T_4	4.23	4.50	4.37	0.28	0.29	0.29
T_5	4.98	5.23	5.11	0.32	0.34	0.33
T_6	4.67	4.77	4.72	0.29	0.30	0.30
T_7	4.03	4.33	4.18	0.27	0.28	0.28
T_8	5.28	5.33	5.31	0.36	0.38	0.37
T_9	4.89	5.37	5.13	0.30	0.34	0.32
T_{10}	5.56	5.80	5.68	0.40	0.42	0.41
Mean	4.30	4.60		0.29	0.31	
Factors	**SE(m) ±**	**C.D. (5%)**		**SE(m) ±**	**C.D. (5%)**	
Media (M)	0.06	0.16		0.004	0.011	
Cow urine (C)	0.02	0.07		0.002	0.005	
M × C	0.08	0.22		0.005	0.015	

The notable increases in relative growth rate (RGR) and total biomass accumulation with cow urine application across various growing media indicate its role in enhancing the physiological growth capacity of papaya seedlings. Cow urine, rich in nitrogenous compounds and growth-promoting hormones, likely facilitated higher metabolic rates, leading to accelerated cell division and elongation (Panchal et al., 2023). Treatments like T_{10} (Soil + Sand + Vermiculite + Cocopeat + Perlite) and T_8 (Soil + Sand + Vermiculite + Perlite), which yielded the highest RGR values, reveal that balanced media mixtures with cow urine can provide optimal aeration, nutrient availability, and moisture retention essential for robust root and shoot growth. The increased biomass accumulation further supports this observation, where cow urine not only supplied necessary nutrients but also promoted enhanced nutrient uptake from the media (Jain et al., 2022). These results were in close agreement with Shinde & Malshe (2015) in *Khirni* when they used cow urine as a seed soak and Sharma et al. (2024) in papaya.

3.3 Leaf Relative Water Content

The results for Leaf Relative Water Content (LRWC) in papaya show a significant improvement with the application of cow urine across different growing media compositions (Fig. 1). In treatments without cow urine, the LRWC ranged from 76.37% in the control treatment (T_0) to a high of 90.0% in T_{10} -Soil + Sand + Vermiculite + Cocopeat + Perlite (1:1:1:1:1), which used a mixture of soil, sand, vermiculite, and cocopeat. When cow urine was applied, LRWC increased in nearly all treatments, with T_{10} -Soil + Sand + Vermiculite + Cocopeat + Perlite (1:1:1:1:1) achieving the highest value of 91.43%, leading to an overall increase in the mean LRWC from 85.75% without cow urine to 87.84% with cow urine. This indicates a positive impact of cow urine on enhancing the water retention ability of papaya leaves across media types. Significantly, the highest LRWC values were observed in treatments T_{10} -Soil + Sand + Vermiculite + Cocopeat + Perlite (1:1:1:1:1) (90.65%), T_9 -Soil + Sand + Vermiculite + Cocopeat (1:1:1:1) (90.25% mean), and T_5 -Soil + Vermiculite + Perlite (1:1:1) (89.62%), suggesting that complex growing media compositions coupled with cow urine application effectively maximize leaf water retention.

The improvement in leaf relative water content (LRWC) with cow urine application demonstrates its role in bolstering the plant's water retention capacity. The increased LRWC in treatments containing cow urine suggests an enhancement in osmotic adjustment and water absorption capacity in the seedlings, an effect that can be crucial under limited water availability (Chakma et al., 2023). Higher LRWC values in treatments with media such as T_{10}, enriched with perlite and cocopeat, are indicative of these materials' ability to retain moisture. The cow urine further contributes to the leaf water status by potentially enhancing root hydraulic conductivity, allowing seedlings to maintain higher tissue hydration, which is essential for sustaining metabolic activities during initial growth phases. These results were closely associated with the findings of Singh et al. (2020) in papaya.

3.4 Proline Content in Leaves

The data on proline content in leaf of papaya indicated a significant effect when cow urine is applied across different growing media compositions (Table 3). For proline content without cow urine, values ranged from 2.57 μM/g fresh weight (FW) in the control treatment (T_0) to 5.57 μM/g FW in T_{10} -Soil + Sand + Vermiculite + Cocopeat + Perlite (1:1:1:1:1), which utilized a mixture of soil, sand, vermiculite, cocopeat and perlite. While, with the cow urine, the proline content further increased, showing the highest value in T_{10} -Soil + Sand + Vermiculite + Cocopeat + Perlite (1:1:1:1:1) (5.70 μM/g FW) and an overall mean rise from 4.31 μM/g FW without cow urine to 4.58 μM/g FW with cow urine. These results highlight that both the type of media and cow urine contribute positively to proline accumulation. Among the treatments, combinations such as T_{10} -Soil + Sand + Vermiculite + Cocopeat + Perlite (1:1:1:1:1) (5.63 μM/g FW), T_9 -Soil + Sand + Vermiculite + Cocopeat (1:1:1:1) (5.35 μM/g FW), and T_5 -Soil + Vermiculite + Perlite (1:1:1) (5.12 μM/g FW) revealed higher proline content.

Proline accumulation in leaves, particularly with cow urine application, suggested an induced biochemical response that may confer stress tolerance (Ahmad et al., 2024). Proline functions as an osmo-protectant and antioxidant, aiding plants in stress mitigation. Its higher accumulation in media enriched with cow urine indicates that cow urine not only acts as a nutrient source but may also trigger stress-response pathways, equipping papaya seedlings with a biochemical advantage in stress management. This is particularly beneficial in environments with variable moisture or nutrient availability, where proline's role in stabilizing proteins and membranes can sustain cell function. Treatments with high proline levels, such as T_{10} and T_9, further underscore the combined effect of balanced media and organic stimulants in fostering resilient seedling physiology. These results were in close agreement with Haryuni et al. (2018) in vanilla and Ansar (2022) in mustard.

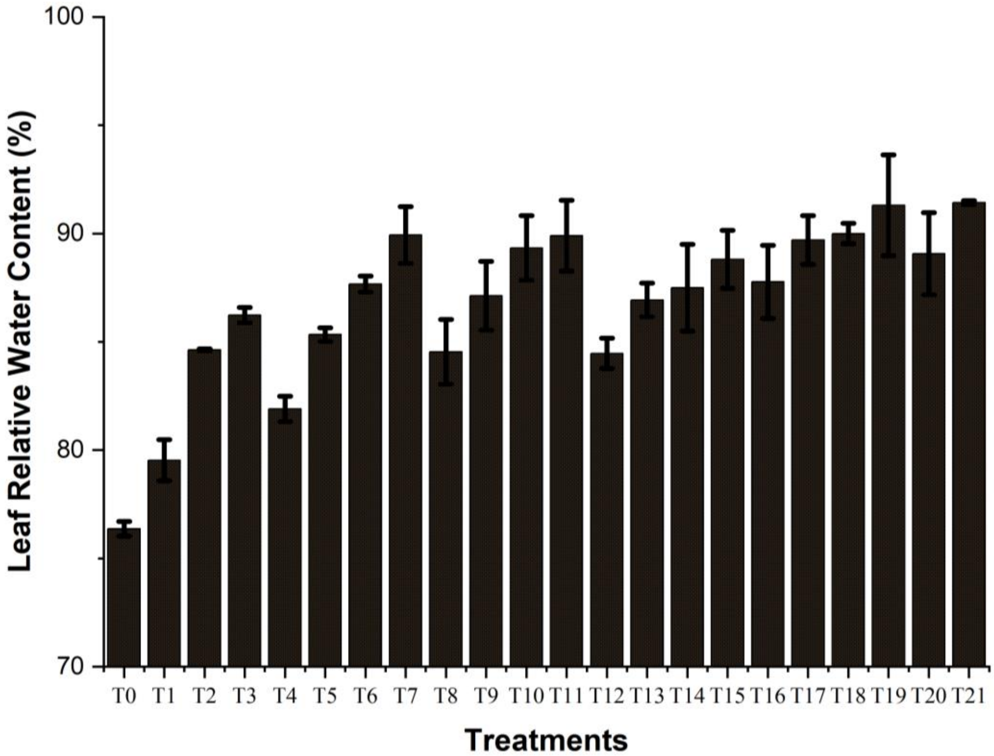

Fig. 1. Effect of growing media and cow urine on leaf relative water content in papaya

Table 3. Effect of growing media and cow urine on proline content in leaf of papaya

Treatments	Proline content in leaf (μM proline g^{-1} FW)		
	Without cow urine	**With cow urine**	**Mean**
T_0 Soil (Control)	2.57	3.27	2.92
T_1 Soil + Sand (1:1)	3.57	3.65	3.61
T_2 Soil + Vermiculite (1:1)	3.40	3.87	3.63
T_3 Soil + Cocopeat (1:1)	4.17	4.23	4.20
T_4 Soil + Perlite (1:1)	4.23	4.50	4.37
T_5 Soil + Vermiculite + Perlite (1:1:1)	5.00	5.23	5.12
T_6 Soil + Vermiculite + Cocopeat (1:1:1)	4.67	4.77	4.72
T_7 Vermiculite + Perlite + Cocopeat (1:1:1)	4.03	4.33	4.18
T_8 Soil + Sand + Vermiculite + Perlite (1:1:1:1)	5.27	5.43	5.35
T_9 Soil + Sand + Vermiculite + Cocopeat (1:1:1:1)	4.90	5.37	5.13
T_{10} Soil + Sand + Vermiculite + Cocopeat + Perlite (1:1:1:1:1)	5.57	5.70	5.63
Mean	4.31	4.58	
Factors	**SE(m) ±**	**C.D. (5%)**	
Media (M)	0.05	0.14	
Cow urine (C)	0.02	0.06	
M × C	0.07	0.20	

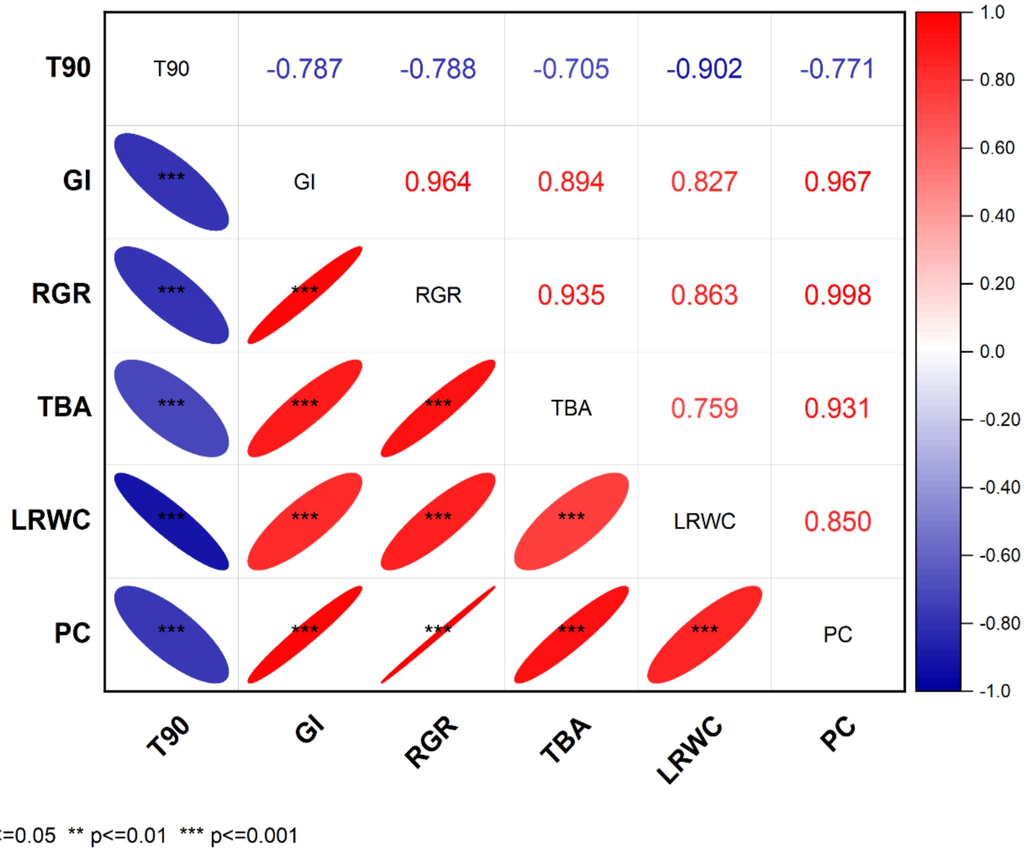

Fig. 2. The different attributes associated with germination, growth and quality of papaya seedling in effect of different growing media and cow urine as revealed using Pearson correlation coefficients

Note: *T90: Time to 90% germination, GI: Germination index, RGR: Relative growth rate, TBA: Total biomass accumulation, LRWC: Leaf relative water content, PC: Proline content in leaf*

3.5 Correlations among Various Parameters under the Investigation

The results showed that proline content in leaves had the highest positive correlation with relative growth rate (r = 0.998), followed closely by its correlation with the germination index (r = 0.967) and total biomass accumulation (r = 0.931). Relative growth rate also had a strong positive correlation with total biomass accumulation (r = 0.935) and leaf relative water content (r = 0.863). Additionally, germination index demonstrated a significant positive correlation with relative growth rate (r = 0.964) and leaf relative water content (r = 0.826), indicating a strong association between seedling vigor, growth rate, and water retention. Whereas for negative correlations, time to 90% germination (T90) showed the maximum negative correlation with leaf relative water content (r = -0.902), followed by with relative growth rate (r = -0.788). The negative correlation between T90 and germination index (r = -0.787) further suggests that faster germination times are associated with improved growth attributes.

The observed strong positive correlations, particularly between proline content and relative growth rate, suggest that proline accumulation is closely linked with vigorous seedling growth. This association may indicate that proline not only serves as a stress marker but also correlates with enhanced growth potential under optimal conditions. Similarly, the high correlation between germination index and relative growth rate supports the idea that rapid and uniform germination leads to better-established seedlings capable of sustaining accelerated growth rates. The negative correlation of time to 90% germination (T90) with LRWC and germination index further implies that faster germination times align with improved growth attributes, as these seedlings are likely to establish stronger water-holding capacity and growth vigor early on.

4. CONCLUSION

This study showed that cow urine, combined with optimized growing media (Soil + Sand + Vermiculite + Cocopeat + Perlite (1:1:1:1:1)), significantly improves germination, growth and stress resilience in papaya seedlings. Reduced time to 90 percent germination (T90) and increased germination index highlight cow urine's stimulatory effects on seed metabolic activity. Enhanced relative growth rate (RGR), total biomass accumulation, and leaf relative water content (LRWC) further affirm cow urine's role in supporting vigorous growth and water retention. Higher proline levels in treated plants suggest improved stress tolerance. These findings indicate that cow urine, as a natural growth enhancer, offers a sustainable means to boost papaya seedling vigor in organic farming.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as large language models (ChatGPT, copilot, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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