Original Research Article

Improvement of Papaya (*Carica papaya* L.) Seed Germination, Seedling Growth and Chlorophyll Content by Using Growing Medium and Organic Liquid

ABSTRACT

A pot experiment was conducted during rainy season (July month) of 2019 at nursery unit of Horticulture Department, College of Agriculture, Ummedganj, Kota to evaluate the impact of growing media and cow urine on seed germination, seedling growth and chlorophyll content of papaya (*Carica papaya* L.) cv. Arka Surya. The experiments were laid out in a factorial complete randomized design with two factors consisting 11 growing media and 2 level of cow urine (with or without). Among different treatments combination used the medium Soil + Sand + Vermiculite + Cocopeat + Perlite (1:1:1:1:1) with cow urine found significantly superior over other treatments and control with respect to lowest days required for first germination (6.17 days) and higher germination percentage (90.24 %). It has given better seedling height (15.48 cm), number of leaves per seedling (9.83) and leaf area (51.85 cm^2) at 75 days after sowing. The maximum length of longest tap root (14.20 cm), diameter of tap root (4.12 mm) and root/shoot ratio (0.87) were also found significantly superior in the same treatment as compared to rest of the treatments. Further, it was also found superior with relation to highest chlorophyll (2.50 mg/100g). Therefore, it is recommended this growing medium combination along with cow urine should be used for better germination and growth of papaya seed by orchardist.

Keywords: *Carica papaya* L.; Growing media; Cow urine; Germination; Growth; Chlorophyll

1. INTRODUCTION

Papaya (*Carica papaya* L.) is a tropical fruit of the family Caricaceae that is highly nutritious and has medicinal properties. In the 16th century, it was introduced to India from tropical America. In current years papaya cultivated in almost every tropical and subtropical country in the world, including Sri Lanka, Tanzania, India, Hawaii, Florida, the Philippines, South Africa and Australia. India has extensively grown it in many states, including Andhra Pradesh, Gujarat, Karnataka, Madhya Pradesh, Maharashtra, West Bengal, Chhattisgarh, Tamil Nadu, Assam and Kerala (Desai *et al*., 2017).

The most beneficial conditions for papaya growth are well-draining and well-aerated soils containing rich organic matter (Morton, 1987). Typically, plants die within 3-4 days in water-logged soils (Storey, 1985). Germination of papaya seeds require 3-5 weeks, and the seeds are enclosed in

a gelatinous sarcotesta. It is important to treat seeds so that they germinate more easily and in less time. Cow urine is a unique product of dairy which has tremendous properties such as being manure, antimicrobial, and disinfectant. There are 95 percent water, 2.5 percent urea, and 2.5 percent enzymes in it (Randhawa and Sharma, 2015). Substrates or growing media are defined as soil materials, other than soil, which, alone or in mixtures, provide better growing conditions than agricultural soil (for one or more aspects). Therefore, media of various origin can provide anchorage for the root system, supply water and nutrients to the plant, and ensure adequate aeration of the root area (Gruda *et al*., 2006; Sharma *et al*., 2021). Accordingly, this study was carried out in order to determine the effect of growing media and cow urine on the seed germination, seedling growth and chlorophyll content of papaya.

2. MATERIALS AND METHODS

A pot experiment on papaya cv. Arka Surya was connected during the month of July in the year 2019 at the nursery unit of the Department of Horticulture, Agricultural University of Kota, Rajasthan which was located in a semi-arid climatic region. The experiment was laid out in factorial complete randomize design with 3 replications. In this experiment consisting of two factors, first factor is the growing media or rooting media and second factor is the cow urine. It was used 11 media combinations with or without cow urine, consisting of total 22 treatments, *i.e.* 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), soil + sand + vermiculite + cocopeat perlite (1:1:1:1:1) (T_{10}). Firstly, a small quantity of cow urine was dissolved in water to prepare the solution of 15%, and then it was mixed with 1 litre of water to achieve the desired concentration. After soaking in cow urine solution for 24 hours, papaya seeds were sown in trays.

The day on which first germination of seed was recorded from the date of sowing. Then the number of days required for first germination was calculated as the days taken for the initiation of germination. The germinated seeds were counted at an interval of two days to last and completion of germination for the calculating germination percentage and the total number of germinated seeds were subtracted from total number of seeds sown and percentage of germination was calculated. The observation pertaining to shoot parameters *viz*. seedling height (cm), number of leaves and leaf area were recorded at 75 days after sowing. The area of each leaf was calculated using a graph paper and expressed as a cm^2. The observation pertaining to root parameters *viz*. length of longest tap root, diameter of tap root and root: shoot ratio were recorded at 75 days after sowing. The length of longest tap root was measured with a meter scale from the point of initiation to the tip. Number of secondary roots were counted after the seedling was uprooted. With a vernier calliper, the diameter of the tap root was measured near the point of initiation. In order to calculate the ratio of root dry weight to corresponding shoot dry weight, used the following formula:

$$\text{Root : shoot ratio} = \frac{\text{Dry weight of roots}}{\text{Dry weight of seedling} - \text{dry weight of roots}}$$

The chlorophyll content in leaves was estimated using dimethyl sulphoxide extraction method (Hiscox and Israelstam, 1979) were estimated at 75 days after sowing and expressed in mg per g.

The data pertaining to various investigations on germination and growth of seedlings were subjected to statistical analysis following Factorial CRD as method as suggested by Panse and Sukhatme (1967). 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 number of days required for first germination and germination percentage of papaya seeds affected significantly by using of different growing media and application of cow urine (Figure 1 and 2). Growing media consisting Soil + Sand + Vermiculite + Cocopeat + Perlite (1:1:1:1:1)-T_{10} showed lower number of days (6.59) required for 1st germination and higher germination percentage (88.22) of seeds as compared to rest of the growing media. While, treatment T_0 (Control) exhibited higher number of days (9.59) for 1st germination and lower germination percentage (73.55) of papaya seeds. The significant impact of cow urine was observed and minimum days required for 1st germination (7.67) and maximum germination percentage (81.50) were noted with cow urine application and maximum days required for 1st germination (8.47) and lower germination percentage (78.01) were registered without application of cow urine. The interaction effect among growing media and cow urine was found statistically significant. The lowest days required for the 1st germination (6.17) and highest germination percentage (90.24) were recorded in treatment T_{10} with cow urine, while, treatment T_0 without cow urine registered highest number of days required for 1st germination (10.00) and lowest germination percentage (72.50). Application of cow urine with growing media consisting Soil + Sand + Vermiculite + Cocopeat + Perlite (1:1:1:1:1)-T_{10} was followed by T_8 for number of days for 1st germination (6.67) and T_9 for germination percentage (88.32) with cow urine. The different growing media and application of cow urine had significant effect on number of days required for 1st germination and germination percentage of papaya seedling. The lowest days required for 1st germination and highest germination percentage were recorded under treatment T_{10} (Soil + Sand + Vermiculite + Cocopeat + Perlite 1:1:1:1:1) with cow urine. The superiority level of germination attributes was noted in soil + sand + vermiculite + vermicompost + cocopeat with cow urine might be due to beneficial effect of all components in improving physical, biological and chemical properties of growing medium as provides natural support, proper aeration and permitting more oxygen which resulted more germination, cocopeat capable for high water holding capacity (Hartmann and Kester, 1997). Retention of more water and air by media helped in quick and early enzymatic action for synthesis of metabolites foe cell multiplication and also enhanced the breakdown of the seed coat resulting in the transformation of embryo into early seedling and which helped for increase germination of seeds (Hasan *et al*., 2010). The cow urine contains physiologically active substances like growth regulators, nutrients (Josef and Nair, 1989) and trace elements (Munoz, 1988) which helped to increase early seed germination and higher germination percentage. Similar results were

observed by Deb *et al*. (2010) and Kumawat *et al*. (2014) in papaya and Patil *et al.* (2012) in rangpur lime.

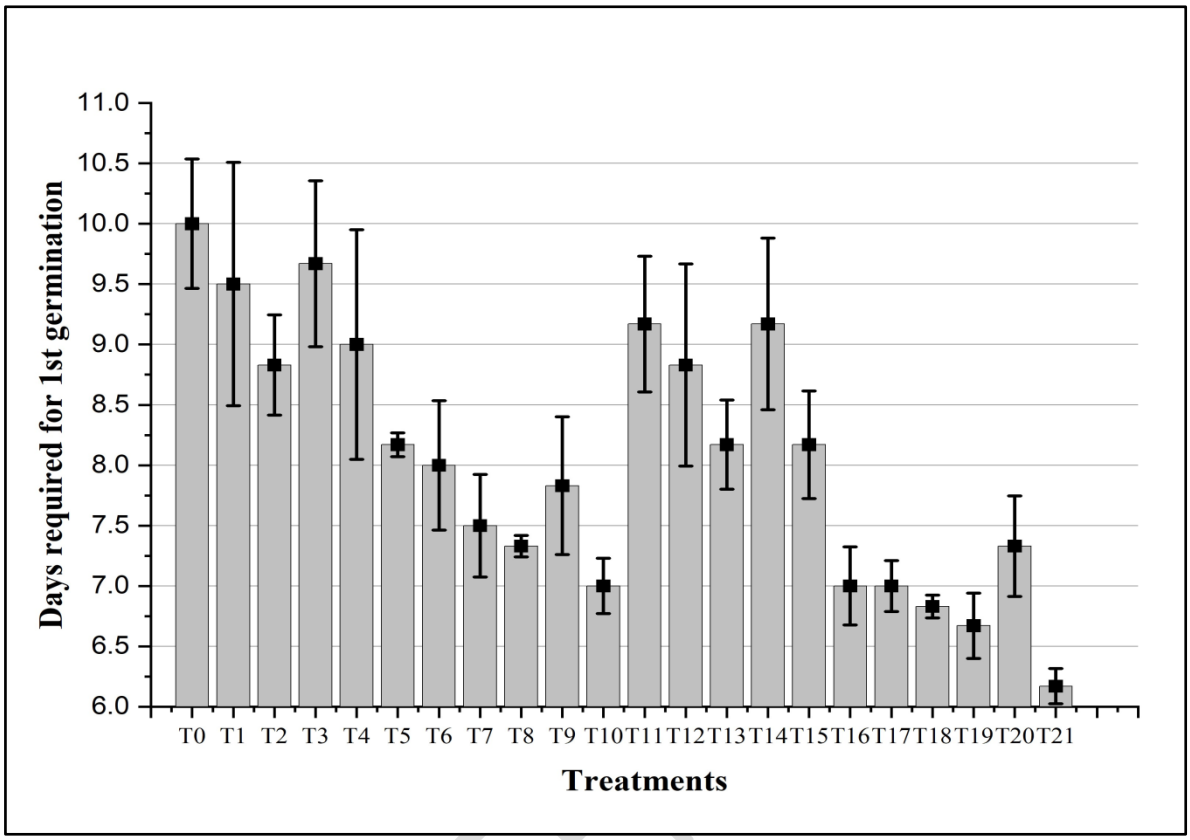


Figure 1: Effect of growing media and cow urine on days required for 1st germination of papaya.

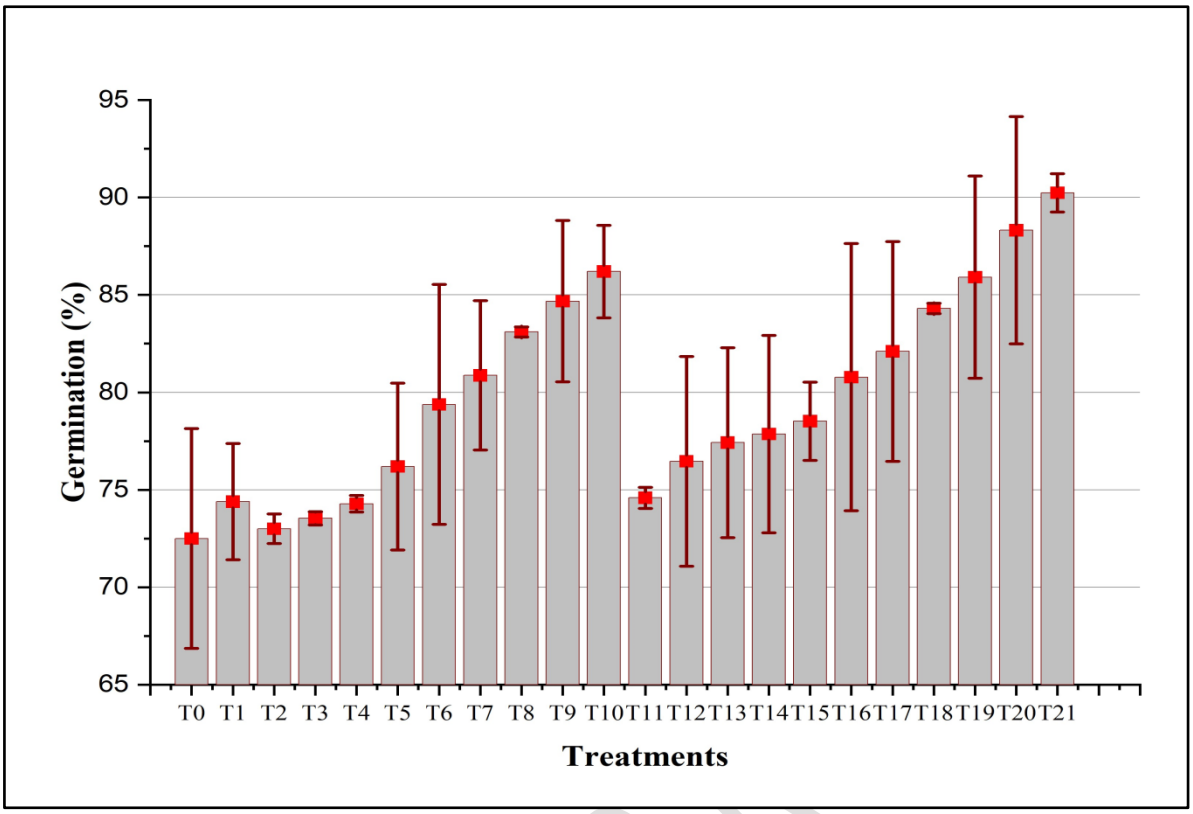


Figure 2: Effect of growing media and cow urine on germination percentage of papaya.

3.2 Seedling growth attributes

Papaya seedling height and number of leaves are affected significantly by use of different growing media and cow urine at 75 days after seed sowing (Figure 3 and 4). In growing media consisting Soil + Sand + Vermiculite + Cocopeat + Perlite (1:1:1:1:1)-T_{10} observed maximum seedling height (14.50 cm) and number of leaves (9.32) as compared to rest of the growing media. However, in T_0 (Control) noted minimum height of seedling (11.24 cm) and number of leaves (6.84). Application of cow urine significantly influenced these growth parameters and registered maximum seedling height (13.50 cm) and number of leaves (8.60) with the application of cow urine, while, minimum seedling height (12.14 cm) and number of leaves (7.46) were observed without application of cow urine. The interaction effect between growing media and cow urine was found statistically significant for seedling height and number of leaves. Maximum seedling height (15.48 cm) and number of leaves (9.83) were recorded in treatment T_{10} with cow urine, while, treatment T_0 without cow urine showed minimum seedling height (10.91 cm) and number of leaves (6.17). Cow urine applied with growing media consisting Soil + Sand + Vermiculite + Cocopeat + Perlite (1:1:1:1:1)-T_{10} was followed by T_9 for seedling height (15.21 cm) and number of leaves (9.67) with cow urine.

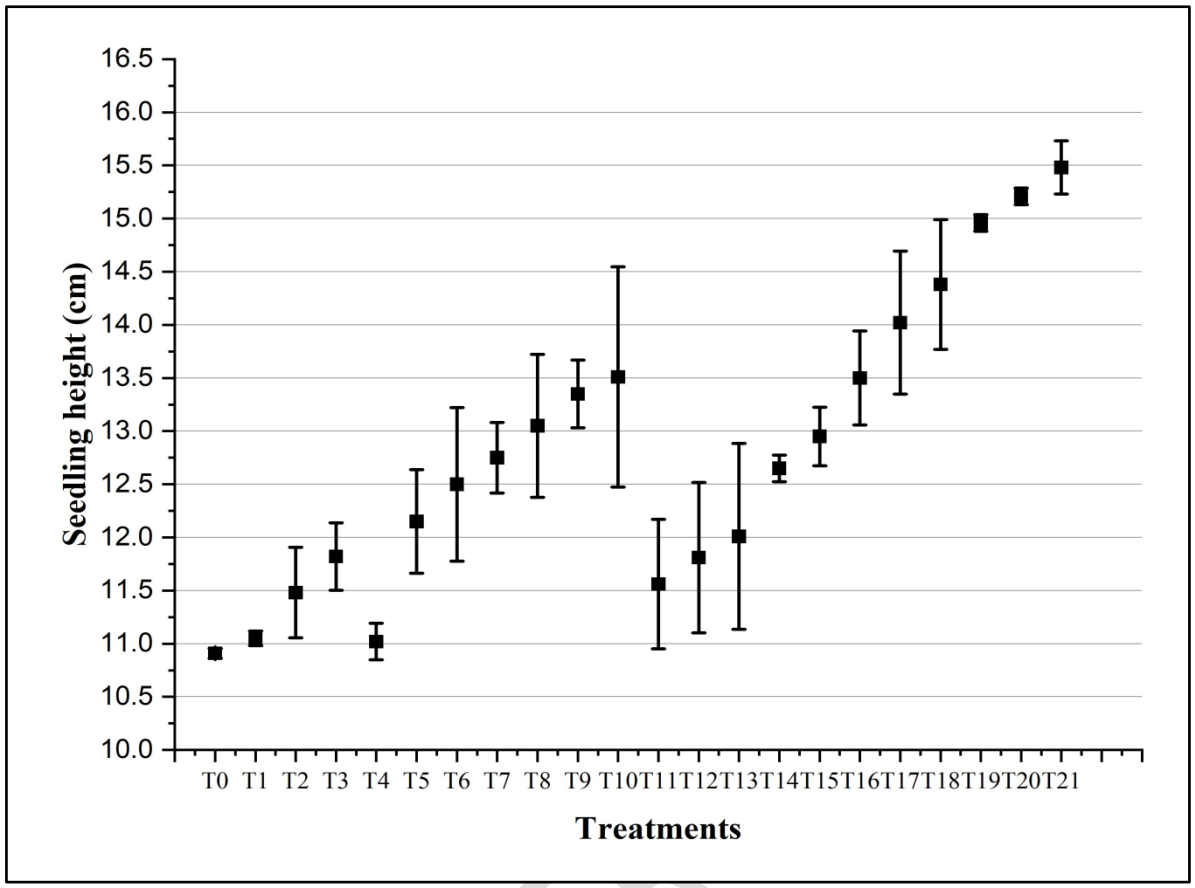


Figure 3: Effect of growing media and cow urine on seedling height of papaya.

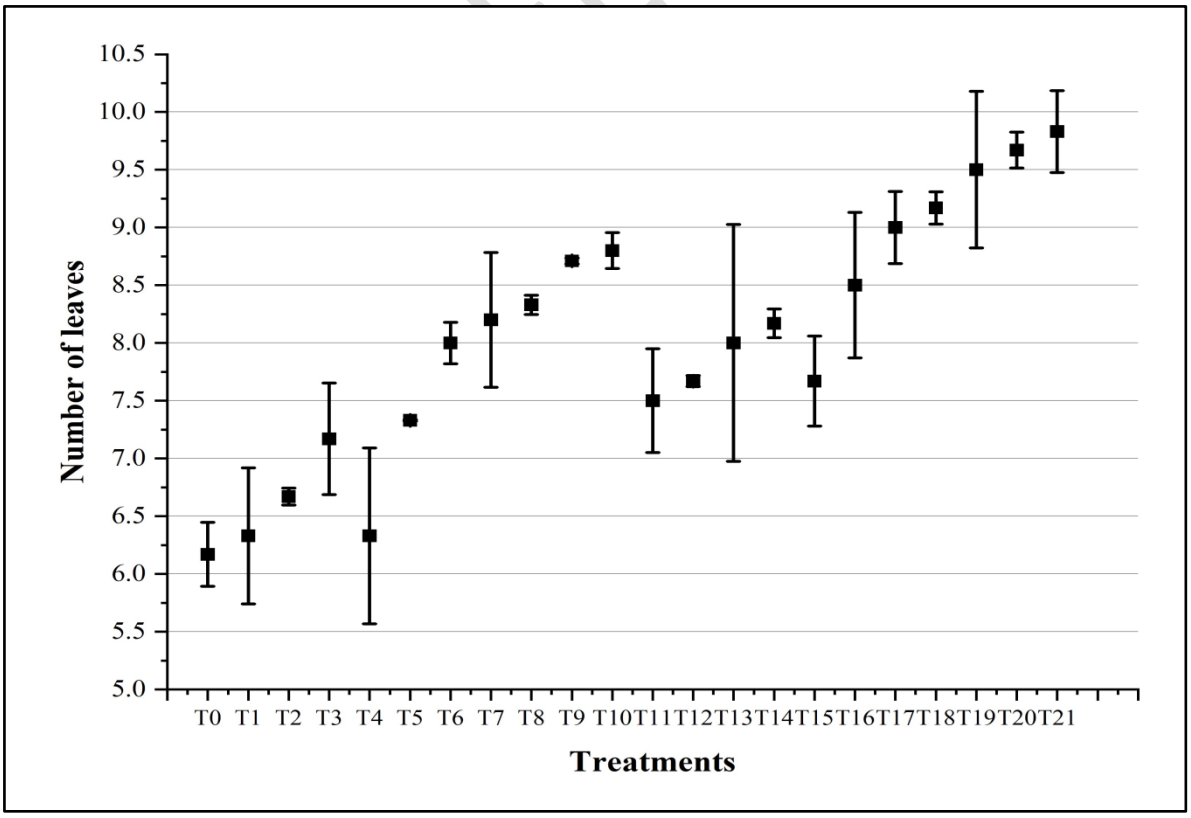


Figure 4: Effect of growing media and cow urine on number of leaves of papaya.

The leaf area of papaya seedlings has been influenced significantly by rooting media and cow urine (Table 1). In treatment T_{10} Soil + Sand + Vermiculite + Cocopeat + Perlite (1:1:1:1:1) observed highest leaf area (49.77 cm^2) and which was followed by T_9 (49.49 cm^2), while, smallest leaf area (36.19 cm^2) was noted in T_0-(Control). Application of cow urine significantly influenced leaf area and registered highest (44.88 cm^2) with cow urine application, while, lowest leaf area (41.45 cm^2) was observed without application of cow urine. Whereas, the interaction effect between growing media and application of cow urine was noted statistically non-significant for leaf area. The different growing/rooting media and cow urine had significant effect on shoot parameters like height of seedling, number of leaves per plant and leaf area of papaya seedling. The maximum seedling height, number of leaves and leaf area were registered under treatment T_{10} (Soil + Sand + Vermiculite + Cocopeat + Perlite 1:1:1:1:1) with the application of cow urine. This might be due to the presence of growth promoting substances (auxins) and nutrients in cow urine and proper media, which leads to better growth of seedlings. Further, the cow urine was maintaining of high water content in cell, increased cell division and cell elongation, which increased the germination and overall growth of the seedlings. The cow urine contains physiologically active substances *viz.,* growth regulators, nutrients (Josef and Nair, 1989) and trace elements (Munoz, 1988) and media provided better condition like aeration and porosity for proper growth and development of seedlings which helped to increase height of the seedlings. These results were in close agreement with Shinde and Malshe (2015) in *Khirni* when they used cow urine as a seed soaking and Sutheesh *et al.,* (2016) in sandalwood.

Table 1: Effect of growing media and cow urine on leaf area of papaya.

Treatments	**Leaf area (cm^2)**		
	Without Cow Urine	**With Cow Urine**	**Mean**
T_0 Soil (Control)	35.16	38.21	36.69
T_1 Soil + Sand (1:1)	36.84	38.98	37.91
T_2 Soil + Vermiculite (1:1)	37.42	41.05	39.24
T_3 Soil + Cocopeat (1:1)	39.65	42.65	41.15
T_4 Soil + Perlite (1:1)	36.50	38.74	37.62
T_5 Soil + Vermiculite + Perlite (1:1:1)	41.26	44.95	43.11
T_6 Soil + Vermiculite + Cocopeat (1:1:1)	43.25	47.12	45.19
T_7 Vermiculite + Perlite + Cocopeat (1:1:1)	44.02	48.23	46.13
T_8 Soil + Sand + Vermiculite + Perlite (1:1:1:1)	46.98	50.12	48.55
T_9 Soil + Sand + Vermiculite + Cocopeat (1:1:1:1)	47.23	51.75	49.49
T_{10} Soil + Sand + Vermiculite + Cocopeat + Perlite (1:1:1:1:1)	47.69	51.85	49.77
Mean	41.45	44.88	
Factors	**SE(m) ±**		**C.D. (5%)**
Media (M)	0.19		0.55
Cow urine (C)	0.46		1.30
M × C	0.64		NS

Length of longest tap root and diameter of papaya seedling were affected significantly by use of different growing media and cow urine (Table 2). In growing media consisting Soil + Sand + Vermiculite + Cocopeat + Perlite (1:1:1:1:1)-T_{10} observed maximum length and diameter (13.68 cm and 3.99 mm, respectively) of tap root as compared to rest of the growing media. However, in T_0 (Control) noted minimum length and diameter (10.22 cm and 3.15 mm, respectively). Application of cow urine significantly influenced these root parameters and registered higher length and diameter (12.26 cm and 3.65 mm, respectively) with cow urine application, while, lower (10.94 cm and 3.26 mm, respectively) were observed in without application of cow urine. The interaction effect between growing media and cow urine was found statistically significant for both root attributes. Maximum length and diameter (14.20 cm and 4.12 mm, respectively) were recorded in treatment T_{10} with cow urine, while, treatment T_0 without cow urine showed minimum length and diameter (9.09 cm and 3.02 mm, respectively) of tap root.

Table 2: Effect of growing media and cow urine on length and diameter of tap root of papaya.

Treatments	Length of longest tap root (cm)			Diameter of tap root (mm)		
	Without Cow Urine	With Cow Urine	Mean	Without Cow Urine	With Cow Urine	Mean
T_0	9.09	11.35	10.22	3.02	3.28	3.15
T_1	9.78	11.67	10.73	3.64	3.61	3.63
T_2	10.83	11.68	11.26	3.18	3.54	3.36
T_3	10.42	11.44	10.93	3.17	3.38	3.28
T_4	10.17	12.67	11.42	3.68	3.86	3.77
T_5	10.65	11.88	11.27	3.14	3.85	3.50
T_6	10.73	11.90	11.32	3.52	3.57	3.55
T_7	11.02	12.35	11.69	3.20	3.53	3.37
T_8	11.86	12.30	12.08	3.20	3.74	3.47
T_9	12.59	13.58	13.09	3.08	3.49	3.29
T_{10}	13.15	14.20	13.68	3.86	4.12	3.99
Mean	10.94	12.26		3.26	3.65	
Factors	**SE(m) ±**	**C.D. (5%)**		**SE(m) ±**	**C.D. (5%)**	
Media (M)	0.13	0.38		0.03	0.09	
Cow urine (C)	0.06	0.16		0.01	0.04	
M × C	0.19	0.53		0.04	0.13	

The use of different growing media with or without cow urine brought out perceptible variation in root/ shoot ratio of papaya seedlings (Figure 5). In growing media consisting Soil + Sand + Vermiculite + Cocopeat + Perlite (1:1:1:1:1)-T_{10} observed maximum root/shoot ratio (0.86) as compared to rest of the growing media. While, in T_0 (Control) noted minimum root/shoot ratio (0.80). Application of cow urine or not was significantly influenced root/shoot ratio and analysed higher (0.84) with cow urine application, while, lower (0.81) was observed in without application of cow urine. The interaction effect between growing media and cow urine was found statistically significant for root/shoot ratio. Maximum root/shoot ratio (0.87) was analysed in treatment T_{10} with cow urine, while, treatment T_0 without cow urine showed minimum (0.78). The different rooting media and cow urine

significantly influenced root characters of papaya seedlings like, length of longest tap root, diameter of tap root and root/shoot ratio. The maximum length of longest tap root diameter of tap root and root/shoot ratio were recorded under treatment T_{10} (Soil + Sand + Vermiculite + Cocopeat + Perlite; 1:1:1:1:1) with the application of cow urine. The beneficial effect on root growth parameters due to rooting media of soil + compost + cocopeat might be due to improved soil texture, structure, porosity, water holding capacity, activity of useful soil micro flora and fauna, maintained soil temperature and improved soil health and nutrient status of medium (Hartmann and Kester, 1997). Further, also provides close contact between seed and rooting media increases steady moisture supply facilitates root respiration and encourages overall root growth (Chatterjee and Choudhari, 2007). It might be due to cow urine was maintaining of high-water content in cell, leads to increase cell division and cell elongation which had increased the overall growth of the seedlings and thereby increased root/shoot ratio. These results were closely associated with the findings of Sutheesh *et al.* (2016) in sandal wood.

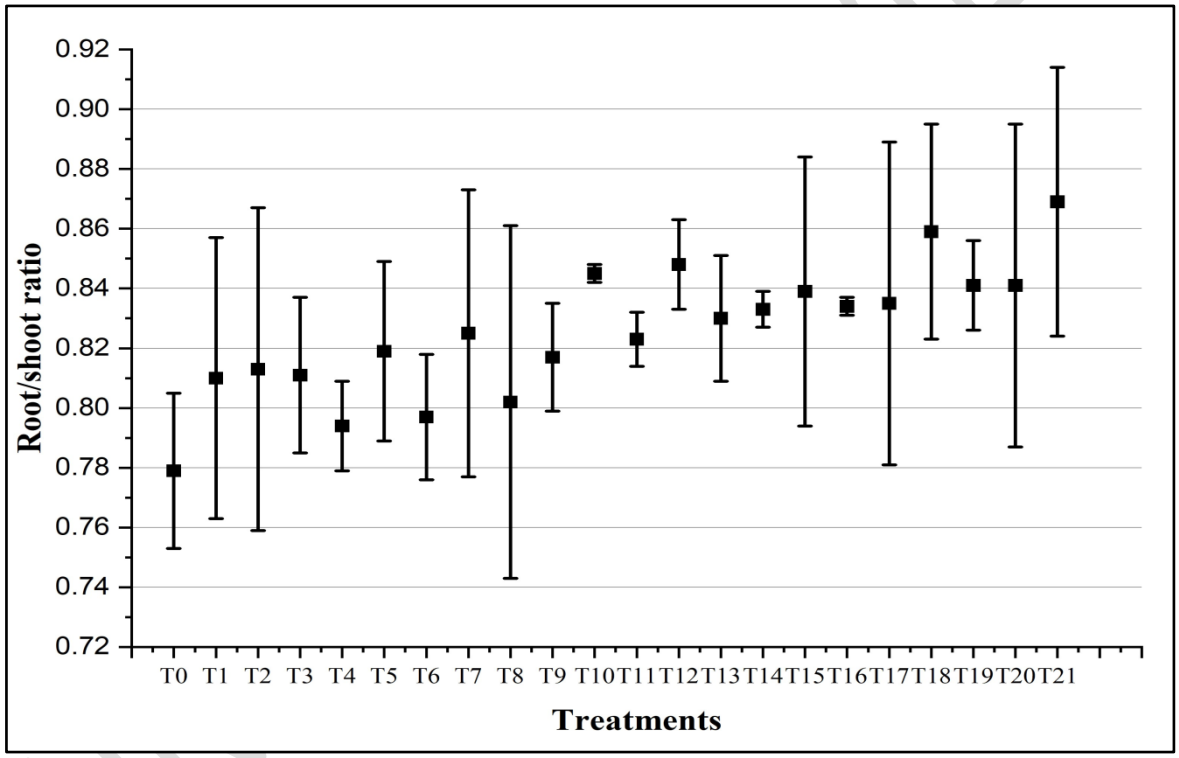


Figure 5: Effect of growing media and cow urine on root/shoot ratio of papaya.

3.3. Chlorophyll content in leaves

Chlorophyll content in leaf of papaya seedling was estimated in relation to various treatments of growing media and cow urine and observed a significant effect (Table 3). In growing media consisting Soil + Sand + Vermiculite + Cocopeat + Perlite (1:1:1:1:1)-T_{10} observed higher chlorophyll content (2.40 mg/g) and it was noted at par with treatment T_9 (2.37 mg/g). While, in T_0 (Control) observed lower chlorophyll content (1.75 mg/g). Application of cow urine or not was significantly influenced chlorophyll content and estimated higher (2.11 mg/g) with cow urine application, while, lower (2.00 mg/g) was observed without application of cow urine. The interaction effect between

growing media and cow urine was found statistically significant for chlorophyll content. Maximum chlorophyll content (2.50 mg/g) was analysed in treatment T_{10} with cow urine, while, treatment T_0 without cow urine showed minimum (1.70 mg/g). The different media and application of cow urine had significant effect on chlorophyll content in leaves. The maximum chlorophyll content in leaves was observed under treatment consisting Soil + Sand + Vermiculite + Cocopeat + Perlite 1:1:1:1:1 with cow urine. This might be due to stimulated nutrient uptake specially nitrogen and synthesis of chlorophyll which have role in the assimilation of numerous amino acids that, are subsequently incorporated in proteins and nucleic acid, which provides framework for chloroplast results into better chlorophyll content in leaves of plant under this treatment (Ramteke *et al.,* 2016).

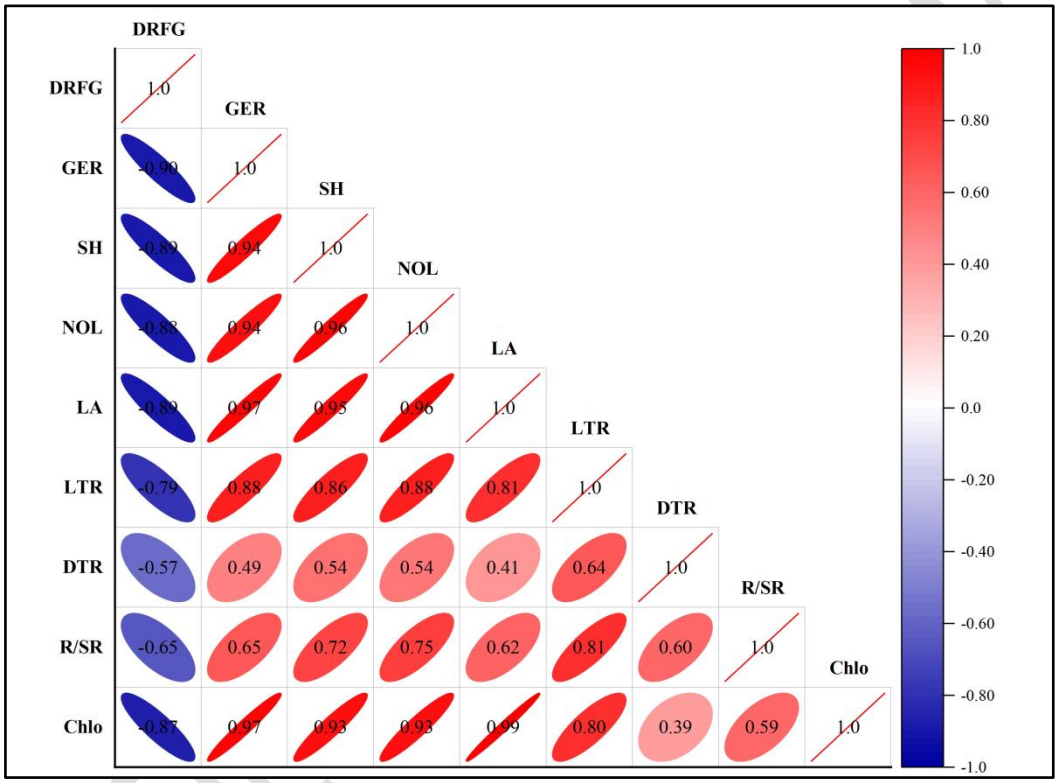
Table 3: Effect of growing media and cow urine on chlorophyll content in leaf of papaya.

Treatments	**Chlorophyll content in leaf (mg/g)**		
	Without Cow Urine	**With Cow Urine**	**Mean**
T_0 Soil (Control)	1.70	1.80	1.75
T_1 Soil + Sand (1:1)	1.78	1.85	1.82
T_2 Soil + Vermiculite (1:1)	1.83	1.93	1.88
T_3 Soil + Cocopeat (1:1)	1.91	2.03	1.97
T_4 Soil + Perlite (1:1)	1.79	1.86	1.83
T_5 Soil + Vermiculite + Perlite (1:1:1)	2.02	2.09	2.06
T_6 Soil + Vermiculite + Cocopeat (1:1:1)	2.09	2.14	2.12
T_7 Vermiculite + Perlite + Cocopeat (1:1:1)	2.14	2.22	2.18
T_8 Soil + Sand + Vermiculite + Perlite (1:1:1:1)	2.19	2.37	2.28
T_9 Soil + Sand + Vermiculite + Cocopeat (1:1:1:1)	2.28	2.45	2.37
T_{10} Soil + Sand + Vermiculite + Cocopeat + Perlite (1:1:1:1:1)	2.30	2.50	2.40
Mean	2.00	2.11	
Factors	**SE(m) ±**		**C.D. (5%)**
Media (M)	0.01		0.02
Cow urine (C)	0.02		0.05
M × C	0.03		0.07

3.4 Correlations among various parameters under the investigation

Significant correlations were observed between different germination, growth and biochemical parameters (Figure 6). The maximum positive correlation was observed between germination percentage and leaf area (0.97); germination percentage and chlorophyll content (0.97) which was followed with good significant positive correlations between seedling height and number of leaves

(0.96); number of leaves and leaf area (0.96); seedling height and leaf area (0.95); germination percentage and seedling height (0.94); and germination percentage and number of leaves (0.94). Germination percentage revealed a significant positive correlation with all parameters excepting days required for 1st germination. There was notable negative correlation was observed between days required for 1st germination with all other parameters. The highest significant negative correlation was observed between days required for 1st germination with germination percentage (-0.90) which was followed by seedling height (-0.89), leaf area (-0.89), number of leaves (-0.88) and chlorophyll content (-0.87).



Note: DRFG: Days required for 1st germination, Ger: Germination (%), SH: Seedling height, NOL: Number of leaves, LA: Leaf area, LTR: Length of tap root, DTR: Diameter of tap root, R/SR: Root/shoot ratio, Chlo: Chlorophyll content

Figure 6: The different attributes associated with germination in effect of different growing media and cow urine on papaya cv. Arka Surya as revealed using Pearson correlation coefficients.

4. Conclusion

On the basis of result obtained from this experiment it may be concluded that the best quality papaya seedlings were obtained when seeds sown in growing medium consisting Soil + Sand +Vermiculite + Cocopeat + Perlite (1:1:1:1:1) and seeds were treated with cow urine. This treatment has given significantly higher germination percentage, seedling height, number of leaves, leaf area, length and diameter of tap root, root/shoot ratio and chlorophyll content in leaf and minimum days required for 1st germination. Therefore, it is recommended that this growing/rooting medium

combination along with cow urine should be used for better germination and growth of papaya seed by orchardist.

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