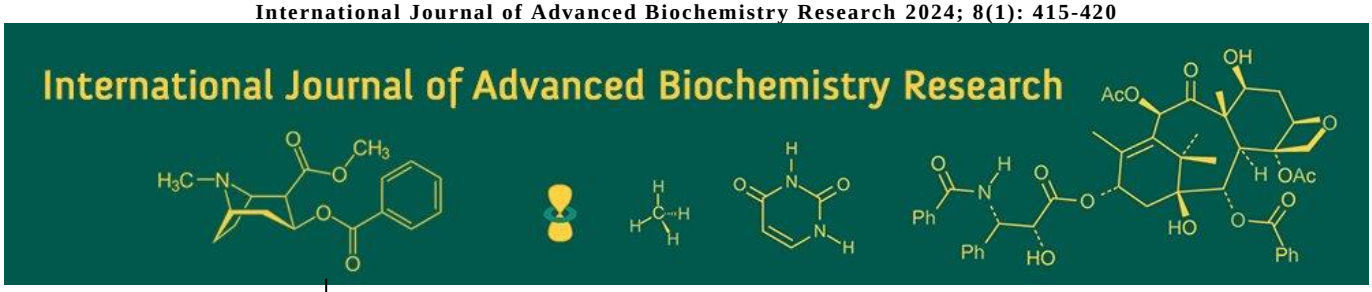

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Effect of pre-sowing treatments on growth of Karonda (*Carissa carandas*) seedlings

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Abstract
The present investigation was conducted at Fruit Research Station, Imalia, Department of Horticulture, College of Agriculture, J.N.K.V.V., Jabalpur (M.P.). The experiment was laid out in Factorial Randomized Block Design with three replications and sixteen treatment combinations. It was carried out to investigate the effect of seed soaking duration (24 hours and 48 hours), growing media (with and without pseudomonas) and concentration of cow urine (0, 25%, 50% and 100%) and their combination on growth of Karonda. The result revealed that treatment T6 with seed soaking duration of 24 hours in 25% cow urine followed by sowing in growing media incorporated with pseudomonas @ 6 ml kg^{-1} soil was found superior with respect to various growth parameters such as seedling length (32.80 cm), length of roots (20.64 cm), seedling vigor index I and II (2764.62 cm & 136.93g), survival percentage (87.66%) over the other treatment combinations.

Keywords: Cow urine, Pseudomonas, Karonda seedlings, growth parameters

Introduction
Carissa carandas is local plant of India whereas 30 species of class Carissa have been detailed by Singh *et al.* (1967) [16]. It is developed all through India in tropical and subtropical ranges. The plants of Carissa are found in fields and slopes and wild frame in Deccan - Promontory as well as in West Coast, Punjab, Kashmir, U.P., Mount Abu (Rajasthan), parts of Gujarat and West Bengal. Karonda could be a natural product of dry regions and thrives well on lands with tall temperature. At display, it is developed on a constrained scale in Rajasthan, Gujarat, Bihar and Uttar Pradesh. It is additionally developed in other nations viz. Bangladesh, South Africa, U.S.A., Denmark, Ghana, Israel, Pakistan, Nepal and Afghanistan. It is additionally found in a few parts of Sri Lanka within the swamp rain woodlands eco-region.
Karonda natural product could be a wealthy source of iron (3.91°per on dry weight iron) and contains a reasonable sum of Vitamin C. Subsequently, it is exceptionally valuable for remedy of frailty and has antiscorbutic properties. Vitamin A substance of natural product is 1619 IU 100 g-1 of consumable parcel as well as 87 to 90% mash, 13 to 14% add up to solvent solids and 4 to 6% acidity. Beneath the changing world exchange situation, it can be utilised on a commercial scale as a natural product for the handling businesses.
The soil media is one of the foremost critical natural variables, which plays a critical part in seedling development and its foundation in early organize. Optimization of plant development by finding reasonable soil media is one of the foremost imperative nursery strategies in raising vigorous seedlings. Beneath ideal conditions, a producer may accomplish not as it were quick uniform germination but too vigorous seedlings with amazing capacity to transport without any check in plant development. Subsequently, growing· media must hold dampness, supply supplements and give back for the seedling. A few supplements must be show, especially phosphorus and calcium. The pH ought to be impartial. Common soil blends, for case break even with parts soil, sand and peat may be utilized. Be that as it may, these have been supplemented to expansive degree by commercially accessible and are utilized in amounts by plant makers. Little seeds require better and more compact medium than is utilized for huge seeds.

Bovine urine contains iron, urea, uric acid, estrogen and progesterone which influence the inhibitory reaction to seed germination, shoot development and seedling vigor (Dilrukshi, 2009) [5].

Plant Growth Promoting Rhizobacteria are the soil microscopic organisms possessing around and, on the surface, and are straight forwardly or by implication included in advancing plant development and improvement through generation and discharge of different administrative chemicals within the region of rhizosphere. They invigorate plant development through mobilizing supplements in soils, creating various plant development controllers, ensuring plants from phytopathogens by controlling or restraining them, moving forward soil structure and bioremediating the contaminated soils by sequestering poisonous overwhelming metal species and corrupting xenobiotic compounds (like pesticides) (Ahemad, 2012; Hayat *et al*., 2010; Rajkumar *et al*., 2010; Braud *et al*., 2009) [1, 7, 12, 4].

Material & Methods

The experiment was carried out at Fruit Research Station, Imalia, JNKVV, Jabalpur. Jabalpur is situated in "Kymore Plateau and Satpura Hills" Agro-climatic zone of Madhya Pradesh at 23.9ºN latitude and 79.58ºE longitude and an altitude of 411.78 meters above the mean sea level. The tropic of cancer passes through the middle of the district.

Climate and weather condition

The climate of Jabalpur locale is semi-arid and subtropical having warm and dry spring summer and cool winter as primary characteristic include, in common the most elevated temperature comes to over 45 °C and underneath 5 °C, within the month of May–June and Dec-Jan separately. The relative mugginess changes from 70-80 percent.

The normal yearly precipitation of approximately 1375 mm, which is primarily conveyed from mid-June to first week of October from south-west storm with incidental rain amid winter.

Treatment Combination

There were sixteen treatment combinations, they are depicted in Table 1.

Table 1: Total treatment combination

Treatments	Treatment Combinations	Composition
T_1	$D_1G_1C_1$	24 hrs. + tap water (control)
T_2	$D_1G_1C_2$	24 hrs + 25% cow urine
T_3	$D_1G_1C_3$	24 hrs + 50% cow urine
T_4	$D_1G_1C_4$	24 hrs + 100% cow urine
T_5	$D_1G_2C_1$	24 hrs + 6 ml per kg + tap water
T_6	$D_1G_2C_2$	24 hrs + 6 ml per kg + 25% cow urine
T_7	$D_1G_2C_3$	24 hrs + 6 ml per kg + 50% cow urine
T_8	$D_1G_2C_4$	24 hrs + 6 ml per kg + 100% cow urine
T_9	$D_2G_1C_1$	48 hrs + tap water
T_{10}	$D_2G_1C_2$	48 hrs + 25% cow urine
T_{11}	$D_2G_1C_3$	48 hrs + 50% cow urine
T_{12}	$D_2G_1C_4$	48 hrs + 100% cow urine
T_{13}	$D_2G_2C_1$	48 hrs + 6ml per kg + tap water
T_{14}	$D_2G_2C_2$	48 hrs + 6 ml per kg + 25% cow urine
T_{15}	$D_2G_2C_3$	48 hrs + 6 ml per kg + 50% cow urine
T_{16}	$D_2G_2C_4$	48 hrs + 6 ml per kg + 100% cow urine

Planning of seed dousing media

25 ml and 50 ml new cow urine was measured with measuring barrel and poured into 100 ml measuring utensil each. The volume was made up by expansion of refined water to plan 25% and 50% arrangement of cow urine separately. 100ml fresh cow urine in 100 ml measuring utensil gave 100% arrangement of cow urine.

Seed treatment

Freshly gathered seeds of Karonda were drenched in cow urine solutions of diverse concentrations for the period of 24 and 48 hours and sown in polybags inside the polyhouse.

Soil treatment

Pseudomonas solution was measured with measuring cylinder and applied to soil @ 6 ml per kg.

Filling of polybags

Polybags of 200 gage thickness having length of 15 cm and breadth of 10 cm were utilized. Sacks were filled as per treatment i.e. with and without pseudomonas.

Seed sowing

Treated seeds of Karonda were sown in polythene sacks of 15 X 10 cm estimate filled with and without Pseudomonas treated soil. One seed per poly pack was sown at 2-2.5 cm profundity. Each treatment was reproduced thrice having 10 polythene packs. Manual water system was connected day by day or as per needs.

Growth Parameters

Seedling height (cm)

After the seed germination five saplings in each treatment and replication were arbitrarily labeled for assist perceptions. The length from the collar locale to the tip of the shoot was measured at 60, 90 and 120 days after sowing for five arbitrarity chosen labeled plants in each treatment and the normal of length was computed.

Stem girth (mm)

The distance across of shoot over the root collar locale was recorded utilizing Vernier caliper at 60, 90 and 120 days after sowing for five arbitrarily chosen labeled plants in each treatment and the normal of size was computed.

Number of leaves per seedling
The total number of leaves were counted from five arbitrarily selected plants in each treatment at 60, 90 and 120 days after sowing and the average of number of leaves was computed.

Length of seedling (cm)
The height of seedlings were measured from root tip to the shoot tip and expressed in centimeter at 120 days after sowing.

Length of root (cm)
The length from collar region to the tip of the root was measured for five randomly uprooted plants in each treatment. The average value was computed.

Number of roots per seedling
The primary, secondary and tertiary roots along with rootlets were counted for the five randomly uprooted plants in each treatment. The average value was computed.

Fresh weight of seedling (g)
The plants were carefully washed to remove the soil adhering to their roots and shoots. The weight was taken with the help of electronic balance and average value was computed.

Dry weight of seedling (g)
For dry weight plant were chopped and oven dried at 60 ± 20C temperature till a constant weight. The weight was taken with the help of electronic balance and average value was computed.

Seedling vigour index I (cm)
It was calculated by adding the values of root length and shoot length which was randomly selected and multiplying with their corresponding germination% and the values were recorded.

Seedling vigour index I = germination% x [root length (cm) + shoot length (cm)]

Seedling vigour index II (g)
It was calculated by multiplying dry weight of seedlings with their corresponding germination%.
Seedling vigour index II = dry weight of seedlings (g) x germination%

Survival percentage of seedlings
The survival percentage of each treatment was recorded at 120 days after seed sowing. The survival percentage was calculated by using formula as given below:

$$\text{Survival of seedling (\%)} = \frac{\text{No. of survived seedling}}{Total\ \text{no. of seedlings}} \text{x } 100$$

Result & Discussion

1. **Seedling height (cm):** The seedling height at 60, 90 and 120 days after sowing, was recorded and presented in Table 2. The interaction of seed soaking duration, growing media and concentration of cow urine has shown significant effect on the height of seedlings at 60, 90 and 120 days after sowing. It was observed that the maximum seedling height at 60, 90 and 120 DAS was attained by seedlings of treatment T6, which was a combination of 24 hrs of seed soaking in 25% cow urine and sowing the seeds in media containing pseudomonas 6ml/kg soil. The recorded seedling height was 9.3, 12.2, 13.1 (cm) at 60, 90, 120 DAS respectively. The interaction of seed soaking duration, growing media and concentration of cow urine showed enhanced seedling height. The findings are in agreements with Sabongari *et al.* (2004) [14] who found that tomato seeds soaked for 24 h recorded the highest stem height. Kloepper *et al.* (1980) isolated two strains of fluorescent pseudomonas from the potato epidermis and celery root significantly increased growth of potato plants up to 500% greater than controls in green house assays.

2. **Stem girth (mm):** The observed maximum stem girth was 2.03, 2.41 and 2.79 (mm) at 60, 90 and 120 DAS respectively obtained in the treatment T6, where seeds were soaked in 25% cow urine for 24 hrs and sown in media containing pseudomonas 6 ml/kg. The findings are in agreement with Pal *et al.* (2019) [11] who conducted an experiment on effect of cow urine and plant growth promoting rhizobacteria (PGPR) on seed germination, growth and survival of Karonda (*Carissa carandas* L.) seedlings and the result evinced that the combined effect of seed treatment with 25% cow urine and soil inoculation with 6ml/kg pseudomonas were more superior over the other combinations with highest seed germination (5.33 days), 50% germination (14.50 days), germination at 60 DAS (79.63%), growth parameters at 60, 90 and 120 DAS with seedling height (8.75, 11.20 and 12.60 cm), stem girth (1.98, 2.36 and 2.64 mm), number of leaves per seedling (29.3, 72.2 and 89.4) respectively, other growth parameters at 120 DAS such as length of seedlings (27.96 cm), seedling vigour index I (2269.7 cm), seedling vigour index II (106.8g), survival of seedlings (81.4%), leaf area index (4.09), leaf area duration (6777.98 cm^2.days), light transmission ratio (34.52%).

3. **No. of leaves per seedling:** Soaking of seeds in 25% cow urine for 24 hrs followed by sowing in growing media treated with Plant Growth Promoting Rhizobacteria produced maximum number of leaves per seedling i.e. 34.4, 83.4 and 101.4 at 60, 90 and 120 DAS respectively. The findings are in agreement with Shivamurthy (2005) [15] recorded significantly higher plant height (74.21 cm), leaf dry weight, more number of tillers (137.4) 60 days after sowing, higher leaf area duration (2.47), higher straw yield (3388 kg per ha) for wheat seeds soaked in 10% cow urine.

4. **Length of seedling (cm):** The maximum length of seedling 32.80 cm at 120 DAS was recorded in treatment T6, the results are in accordance with the findings of Rao (1975) [13] who reported that seed soaking of custard apple in 10% cow urine for 24 hours increased germination percentage, height of seedling, no. of leaves per seedlings, number of roots, length of roots and fresh and dry weight of seedlings as compared to control. Vijayalakshmi *et al.* (1998) [19] prepared

mixture of 50 g of powdered rhizomes 2.5 l of water 1 l of cow urine cooking pat basin for treatment of 1 kg seeds. Sweet flag seeds treated with the solution reported high germination, fast seedling growth and also increased tolerance against insect pest.

5. **Length of roots (cm) & number of roots per seedling:** The maximum length of roots and number of roots per seedling was obtained in treatment T6 i.e. 20.64 cm and 130.16 respectively. The findings are in agreement with Gupta (1988) [6], who treated the seeds of Ber (*Ziziphus mauritiana* Lam.) with water dipping + dipping in IPA 500 ppm for 36 hours + 24 hours dipping in cow urine + dipping in IPA 500 ppm for 36 hours, dipping in concentrated H_2SO_4 for 5 minutes + dipping in IPA 500 ppm for 5 minutes + 24 hours dipping in IPA (indole - 3- propionic acid) 500 ppm, dipping in concentrated H_2SO_4 for 5 minutes and control. He concluded that the overall finding of his study revealed that the application of conc. H_2SO_4 + IPA followed by IPA and cow urine helped early germination and showed best results at 50 percent germination and completing the germination, increasing growth attributes of ber namely height of plant, branches per plant, leaves per plant, diameter of stem, length of root, control showed poorest result.

6. **Fresh and dry weight of shoots (g):** Maximum fresh and dry weight of shoots was obtained in treatment T6 i.e. 2.85 g & 1.39 g respectively. The findings are in accordance with who soaked *Albizia lebbeck* seeds in cow urine and observed maximum shoot length, root length, total leaf area and total dry weight of seedlings as compared to unsoaked seeds. Suslow and Schroth (1982) [18] selected strains of fluorescent Pseudomonas spp. isolated from the rhizosphere or rhizoplane of field grown sugar beets caused statistically significant yield increase of sugar beets in replicated greenhouse studies, when applied as seed coating formulation. The increase in fresh and dry weight of roots and shoots of plants grown in greenhouse ranged from 20 to 85%.

7. **Fresh & dry weight of roots (g):** The maximum fresh & dry weight of roots was recorded in treatment T6 i.e. 0.94 g & 0.43 g respectively. The observations are in agreement with Sitinjak (2018) [17] who carried out an experiment to study about the potential of immature coconut water and cow urine on the growth of palm oil seedlings in pre-nursery. The experiment consisted of two factors, first immature coconut water with 4 levels, viz 0 ml (K_0), 10 ml (K_1), 20 ml (K_2), and 40 ml (K_3) and the second factor was cow urine with the levels of 0.0 (U_0), 10 ml (U_1), 20 ml (U_2), and 40 ml (U_3) and the results revealed that the application of immature coconut water and naturally fermented cow urine significantly affected the seedling height, leaf length, stem diameter, primary root length, fresh weight and dry weight of oil palm seedlings at the pre- nursery stage. The combined application of 40 ml of coconut water and 20 ml of cow urine significantly increased the growth (seedling height, leaf length, stem diameter, and fresh weight) of oil palm seedlings at the pre-nursery. Vrany and Fiker (1984) [20] conducted pot culture experiments to know the effects of isolates of *Pseudomonas fluorescens* on the growth of potato plants and their tubers. Inoculation of tuber pieces with the isolates caused a better growth of potato plants. When nutrient solutions used for the growth promoting Pseudomonas strains, revealed an increase in root and shoot fresh weight and was correlated to the suppression of deleterious rhizosphere microorganisms.

8. **Seedling Vigor Index I & II:** Highest Seedling Vigor Index I (2764.62 cm) and highest Seedling Vigor Index II (136.93 g) was observed in treatment T6. The results are in accordance with Ambika *et al.* (2014) [3] who studied the effect of pre sowing seed treatments with urines of cow, buffalo, sheep, goat and pig. They soaked the seed of paddy, maize, sorghum, *Cumbu irungucholam* seeds (local type) for 3 hrs. with concentrations of 5 and 10% along with control (dry treatment). The seed quality parameters viz., earliness of germination, germination percentage, seedling length (cm) and vigour index were evaluated and found among the bovine urine treatments the best performance was recorded in cow urine (5 percent) in all the cereals. Ahmad (2013) [2] proved that Rhizobium and Pseudomonas ACC-deaminase-producing strains can improve the growth, physiology, and quality of mung beans under salt-affected environments.

9. **Survival percentage of seedlings:** The maximum survival percentage was observed in treatment T6 with 87.66%. The findings are in accordance with Yuen and Schroth (1986) [21] who observed that *Pseudomonas fluorescens* E – 6 increased the growth of carnation, sunflower, vinca and zinnia than those of controls. When inoculated onto the seeds or rooted cuttings. Ozturk *et al.* (2003) [10] reported that plant growth promoting rhizobacteria enhanced yield and growth of wheat.

Table 2: Effect of pre sowing treatments on growth parameters of Karonda seedlings

Treatment combination	Seedling height (cm)			No. of leaves per seedling			Stem girth (mm)		
	60 DAS	90 DAS	120 DAS	60 DAS	90 DAS	120 DAS	60 DAS	90 DAS	120 DAS
$D_1G_1C_1$	4.2	7.0	9.6	12.4	31.7	48.6	1.33	1.82	2.10
$D_1G_1C_2$	7.0	9.2	11.2	22.6	50.1	62.1	1.83	2.13	2.33
$D_1G_1C_3$	7.0	9.1	11.0	21.4	49.0	62.2	1.74	2.04	2.30
$D_1G_1C_4$	6.5	9.1	10.9	21.0	48.8	60.6	1.74	2.03	2.27
$D_1G_2C_1$	6.1	8.1	10.0	19.9	40.6	51.0	1.71	1.93	2.17
$D_1G_2C_2$	9.3	12.2	13.1	34.4	83.4	101.4	2.03	2.41	2.79
$D_1G_2C_3$	8.5	10.3	12.1	32.4	78.2	84.7	2.00	2.32	2.59
$D_1G_2C_4$	8.0	10.2	12.1	25.0	71.3	79.6	1.94	2.32	2.54
$D_2G_1C_1$	5.6	7.6	9.0	13.8	32.4	50.0	1.50	1.91	2.16
$D_2G_1C_2$	6.4	8.8	10.9	20.8	42.7	52.8	1.73	2.01	2.23

$D_2G_1C_3$	6.3	9.0	10.0	20.6	41.6	53.5	1.74	1.95	2.20
$D_2G_1C_4$	6.2	8.2	10.1	20.3	40.4	52.3	1.71	1.94	2.21
$D_2G_2C_1$	6.1	8.0	9.8	18.6	40.8	50.8	1.59	1.91	2.13
$D_2G_2C_2$	8.1	10.1	12.0	24.2	61.0	77.4	1.84	2.32	2.49
$D_2G_2C_3$	7.1	10.1	11.2	23.8	59.1	71.2	1.83	2.24	2.42
$D_2G_2C_4$	7.1	9.3	11.1	23.0	53.8	67.9	1.82	2.15	2.34
SEm±	0.05	0.07	0.12	0.41	1.06	0.64	0.01	0.005	0.009
CD at 5% level	0.16	0.22	0.37	1.19	3.08	1.87	0.03	0.01	0.02

Table 3: Effect of pre sowing treatments on growth parameters at 120 DAS of Karonda seedlings

Treatment combinations	Fresh weight of shoots (g)	Dry weight of shoots (g)	Fresh weight of roots (g)	Dry weight of roots (g)
$D_1G_1C_1$	1.08	0.34	0.34	0.13
$D_1G_1C_2$	2.15	0.71	0.54	0.22
$D_1G_1C_3$	2.03	0.74	0.48	0.20
$D_1G_1C_4$	2.01	0.65	0.48	0.19
$D_1G_2C_1$	1.38	0.48	0.40	0.14
$D_1G_2C_2$	2.85	1.39	0.94	0.43
$D_1G_2C_3$	2.63	0.93	0.86	0.27
$D_1G_2C_4$	2.46	0.86	0.63	0.24
$D_2G_1C_1$	1.14	0.37	0.36	0.14
$D_2G_1C_2$	1.82	0.64	0.46	0.18
$D_2G_1C_3$	1.71	0.56	0.45	0.17
$D_2G_1C_4$	1.52	0.53	0.44	0.17
$D_2G_2C_1$	1.26	0.38	0.31	0.15
$D_2G_2C_2$	2.40	0.80	0.60	0.25
$D_2G_2C_3$	2.37	0.79	0.55	0.24
$D_2G_2C_4$	2.34	0.78	0.55	0.24
SEm±	0.05	0.05	0.001	0.004
CD at 5 percent level	0.15	0.16	0.003	0.01

Table 4: Effect of pre sowing treatments on growth parameters at 120 DAS of Karonda seedlings

Treatment combinations	Length of seedling (cm)	Length of roots(cm)	Number of roots per seedling
$D_1G_1C_1$	18.81	12.20	39.53
$D_1G_1C_2$	23.12	18.31	81.26
$D_1G_1C_3$	21.54	18.51	75.53
$D_1G_1C_4$	20.74	17.34	60.33
$D_1G_2C_1$	19.99	14.87	48.33
$D_1G_2C_2$	32.80	20.64	130.16
$D_1G_2C_3$	30.81	20.60	127.46
$D_1G_2C_4$	27.90	20.10	115.26
$D_2G_1C_1$	20.02	12.12	40.93
$D_2G_1C_2$	20.71	16.37	59.56
$D_2G_1C_3$	20.26	15.85	51.73
$D_2G_1C_4$	20.15	14.95	51.20
$D_2G_2C_1$	19.95	12.92	45.26
$D_2G_2C_2$	23.98	19.91	106.60
$D_2G_2C_3$	23.65	19.85	103.00
$D_2G_2C_4$	23.45	19.37	88.40
SEm±	0.24	0.27	1.67
CD at 5 percent level	0.70	0.80	4.80

Table 4: Effect of pre sowing treatments on growth parameters at 120 DAS of Karonda seedlings

Treatment combinations	Seedling vigour index I (cm)	Seedling vigour index II (g)	Survival percentage of seedlings
$D_1G_1C_1$	1146.23	30.45	63.06
$D_1G_1C_2$	1616.26	66.33	69.90
$D_1G_1C_3$	1405.96	61.24	69.20
$D_1G_1C_4$	1364.83	56.70	67.93
$D_1G_2C_1$	1275.59	42.70	66.36
$D_1G_2C_2$	2764.62	136.93	87.66
$D_1G_2C_3$	2584.29	101.21	86.36
$D_1G_2C_4$	2096.44	82.06	76.06
$D_2G_1C_1$	1267.77	32.98	65.50
$D_2G_1C_2$	1333.92	54.88	67.80
$D_2G_1C_3$	1287.09	45.95	67.10
$D_2G_1C_4$	1278.29	45.92	66.36

$D_2G_2C_1$	1224.62	34.47	64.20
$D_2G_2C_2$	1774.95	76.76	75.26
$D_2G_2C_3$	1658.94	75.06	74.00
$D_2G_2C_4$	1637.19	73.02	72.20
SEm±	30.71	1.24	0.52
CD at 5 percent level	88.72	3.59	1.50

Conclusion

The result of the present investigation reveals that seed soaking duration (24 hours), concentration of cow urine (25%) and growing media (incorporation of Pseudomonas @ 6 ml kg^{-1} soil) applied alone or in different combinations were found superior with respect to considerable increase in the seedling height, number of leaves per seedling, stem girth, length of seedlings, length of roots, fresh & dry weight of shoots, fresh & dry weight of roots, number of roots per seedling and led to the enhancement in growth and survivability of Karonda seedlings.

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