Influence of seed soaking duration and concentration of cow urine on seed germination, growth and survival of Karonda (Carissa carandas L.) seedlings

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Abstract
Field experiment was conducted at Fruit Research Station, Imalia, Department of Horticulture, College of Agriculture, J.N.K.V.V., Jabalpur (M.P.) during the year 2018 – 2019 to determine the influence of seed soaking duration and concentration of cow urine on seed germination, growth and survival of Karonda (Carissa carandas L.) seedlings. The experiment was laid out in eight treatments replicated thrice in factorial randomized block design. Among the treatments, the combined effect of seed soaking duration for 24 hours and seed treatment with 25% cow urine was more superior over the other combinations with highest seed germination (5.83 days), 50% germination (15.50 days), germination at 60 DAS (78.15%), coefficient of germination (5.83), speed of germination (8.61). Growth parameters at 60, 90 and 120 DAS with seedling height (8.21, 10.74 and 12.20 cm), stem girth (1.93, 2.27 and 2.56 mm), number of leaves per seedling (28.5, 66.7 and 81.7) respectively, other growth parameters at 120 DAS such as length of seedling (27.96 cm), number of roots per seedling (105.7), fresh weight of seedlings (3.24g), dry weight of seedlings (1.38g), seedling vigor index I (2190.4 cm), seedling vigor index II (101.6g), survival of seedlings (78.7%), leaf area index (3.75), leaf area duration (618.45 cm², days), light transmission ratio (36.28%) and energy interception (0.40 cal cm⁻² min⁻¹).

Keywords: Karonda (Carissa carandas L.), cow urine, soaking duration, seed vigor

Introduction
Carissa carandas is native plant of India while 30 species of genus Carissa have been reported by Singh et al. (1967) [7]. It is cultivated throughout India in tropical and subtropical areas. The plants of Carissa are found in plains and hills and wild in Deccan - Peninsula as well as in West Coast, Punjab, Kashmir, U.P., Mount Abu (Rajasthan), parts of Gujarat and West Bengal. Karonda is a fruit of dry areas and flourishes well on lands with high temperature. At present, it is grown on a limited scale in Rajastan, Gujarat, Bihar and Uttar Pradesh. It is also grown in other countries viz. Bangladesh, South Africa, U.S.A., Denmark, Ghana, Israel, Pakistan, Nepal and Afghanistan. It is also found in some parts of Sri Lanka in the lowland rain forests eco-region. Karonda fruit is a rich source of iron (3.91°per on dry weight basis) and contains a fair amount of Vitamin C. Therefore, it is very useful for cure of anemia and has antiscorbutic properties. Vitamin A content of fruit is 1619 IU 100 g⁻¹ of edible portion as well as 87 to 90 per cent pulp, 13 to 14 per cent total soluble solids and 4 to 6 per cent acidity. Under the changing world trade scenario, it can be exploited on a commercial scale as a fruit for the processing industries. Since, Karonda is very hardy and drought tolerant fruit crop but heavy rainfall and water logged conditions are not desirable. The roots of the plant are heavily branched and makes it suitable for stabilizing eroding slopes. It can be grown on a wide range of soils including saline and sodic soils. Propagation by seed is the common method by which plant reproduce in nature and one of the most efficient and widely used propagation methods for raising seedling. Sowing of seed is the physical beginning of seedling propagation. Specific physical and chemical changes that take place during maturation and ripening of the fruit lead to fruit senescence and dissemination of the seed. One of the most obvious changes is the drying of the pericarp tissues. In certain species, this lead to dehiscence and the discharge of the seeds from the fruit.
Seeds of most species dehydrate at ripening and prior to dissemination, when moisture content drop to 30 per cent or less. The seed dries further during harvest, usually to about 4-6 per cent for storage. Germination cannot take place at this level of dryness which is important basis for maintaining viability and controlling germination. In certain species, seed must not dry below which 30 to 50 per cent or they will lose their ability to germinate (Chin 1981) [1]. Plant species can be separate as recalcitrant or orthodox seed based on their genetic potential to tolerate storage. Most recalcitrant seed cannot tolerate moisture below 25 per cent and some species are also sensitive to chilling temperature. Karonda is commonly propagated by seed and fresh seeds are sown for raising seedlings in the month of August and September. Cow urine contains iron, urea, uric acid, estrogen and progesterone which affect the inhibitory response to seed germination, shoot growth and seedling vigor (Dilrukshi, 2009) [2]. The optimal level of soaking have enhanced effect on germination and growth, due to breakdown of complex into simpler sugars that are readily utilized in the synthesis of auxins and proteins. The auxins produced help to soften cell walls to facilitate growth and the proteins readily utilized in the production of new tissues.

Materials and Methods
An experimental study on “Influence of seed soaking duration and concentration of cow urine on seed germination, growth and survival of Karonda (Carissa carandas L)” was conducted at Fruit Research Station, IMalia, Department of Horticulture, College of Agriculture, J.N.K.V.V., Jabalpur (M.P.) during the year 2018 – 2019. There were eight treatment combinations namely T0 (Control), T1 (24 hours soaking in 25% cow urine), T2 (24 hours soaking in 50% cow urine), T3 (24 hours soaking in 100% cow urine), T4 (48 hours soaking in water), T5 (48 hours soaking in 25% cow urine), T6 (48 hours soaking in 50% cow urine), T7 (48 hours soaking in 100% cow urine). The experiment was laid out in poly bags in factorial randomized block design with three replications.

Seed Vigor Parameters
Initiation of germination
The days taken to initiate germination in each treatment after sowing were observed and recorded.

50% germination
The days taken for 50 per cent germination in each treatment after sowing were observed and recorded.

Germination at 60 days after sowing (%)
The germination in each treatment was recorded at 60 days after sowing. Number of germinated seeds were counted and expressed as germination percentage.

Coefficient of germination
The germination in each treatment was recorded after sowing. Number of seedlings were counted and expressed the coefficient of germination.

Speed of germination
When seeds started germinating their number was counted daily till maximum germination was obtained.

Growth Parameters
Seedling height
Five plants were randomly selected for recording the shoot length of seedlings. They were measured by using centimeter scale and recorded at 60, 90 and 120 DAS.

Stem girth (mm)
The diameter of shoot above the root collar region was recorded using Vernier caliper at 60, 90 and 120 days after sowing for five randomly selected tagged plants in each treatment and the average of girth was computed.

Number of leaves per seedling
The total number of leaves were counted from five randomly 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 seedling is measured from root tip to the shoot tip and expressed in centimeter at 120 days after sowing.

Number of roots per seedling
The number of roots per seedling, primary, secondary and tertiary and rootlets were counted.

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 plants were chopped and oven dried at 60 ± 20°C temperature till a constant weight. The weight was taken with the help of electronic balance and average value was computed.

Seedling vigor 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 per cent and the values were recorded.

Seedling vigor index I = germination per cent x [root length (cm) + shoot length (cm)]

Seedling vigor index - II (g)
It was calculated by multiplying dry weight of seedlings with their corresponding germination per cent.

Seedling vigor index II = dry weight of seedlings (g) x germination per cent

Survival percentage of seedlings
The survival percentage of each treatment was recorded at 120 days after seed sowing.

Physiological Parameters
Leaf Area Index
LAI expresses the ratio of leaf surface considerably to the ground area occupied by the plant or a crop stand worked out as per specification of Gardner et al. (1985)

Leaf Area Duration (LAD) (cm², days)
Leaf Area Duration expresses the magnitude and persistence of leaf area of leafiness during the period of crop growth. It reflects the extent of period of crop growth. It reflects the extent of seasonal integral of light interaction and corrected with yield.

Light Transmission Ratio (LTR) (%)
It was given by Golingai and Mabbayad in 1969.
Energy Interception (EI) (cal cm² min⁻¹)
It can be calculated by lux meter. Its values were converted in terms of energy as per constants. The values were recorded at 90 and 120 DAS and then the mean value was worked out. 71 K Lux =1 calorie per cm² per min
EI = total incident energy – transmitted energy

Results and Discussion
Seed Vigor Parameters
The results on the effect of seed soaking duration and concentration of cow urine on seed vigor parameters is shown in Table 1. T₁ (24 hours soaking in 25% cow urine) showed early germination (5.83), minimum days taken to 50% germination (15.50), high speed of germination (8.61), high coefficient of germination (5.83) and high percentage of germination (78.15), whereas T₀ (Control) recorded maximum days for initiation of germination (9.50) and lowest speed of germination (5.31) while T₄ (48 hours soaking in water) showed maximum days taken to 50% germination (28.83), lowest germination percentage (63.93) and lowest coefficient of germination (4.12). The findings are supported by Parameswari et al. (2001) [4].

Growth Parameters
The results on the effect of seed soaking duration and concentration of cow urine on growth parameters is shown in Table 1, 2 and 3. T₁ (24 hours soaking in 25% cow urine) significantly increased the height (8.21, 10.74 and 12.20 cm) of seedlings, stem girth (1.93, 2.27 and 2.56 mm), number of leaves per seedling (28.5, 66.7 and 81.7) at 60, 90 and 120 days after sowing, number of roots per seedling (105.7), length (27.96 cm) of seedling, fresh weight of seedlings (3.24g), dry weight of seedlings (1.38g), seedling vigor index I (2190.4 cm), seedling vigor index II (101.6g), survival percentage (78.78) at 120 days after sowing. The findings are supported by Rao (1975) [5].

Physiological Parameters
The results on the effect of seed soaking duration and concentration of cow urine on growth parameters is shown in Table 3. Treatment combination T₁ (24 hours soaking in 25% cow urine) recorded maximum leaf area index (3.75), leaf area duration (6181.45 cm² days), energy interception (0.40 cal cm² min⁻¹) and least light transmission ratio (36.28%). Whereas, T₀ (Control) recorded minimum leaf area index (1.25), leaf area duration (1904.31 cm² days), energy interception (0.12 cal cm² min⁻¹) with highest light transmission ratio (76.30%). The findings are supported by Illango et al. (1999) [3].

Table 1: Influence of seed soaking duration and concentration of cow urine on seed vigor and growth parameters

| Treatments | Initiation of germination (days) | 50% germination (days) | Germination 60 DAS (%) | Speed of germination | Coefficient of germination | Seedling height (cm) |
|------------|---------------------------------|-----------------------|------------------------|----------------------|--------------------------|----------------------|
| T₀ - control (tap water) | 9.50 | 23.50 | 64.35 | 5.13 | 5.83 | 8.21 | 10.74 | 12.20 |
| T₁ – 24 hours soaking in 25% cow urine | 5.83 | 15.50 | 78.15 | 8.61 | 5.83 | 8.21 | 10.74 | 11.60 |
| T₂ – 24 hours soaking in 50% cow urine | 6.67 | 16.00 | 77.48 | 8.38 | 5.40 | 7.78 | 9.74 | 11.60 |
| T₃ – 24 hours soaking in 100% cow urine | 7.41 | 16.50 | 71.75 | 7.76 | 5.20 | 7.31 | 9.69 | 11.54 |
| T₄ – 48 hours soaking in tap water | 7.33 | 23.83 | 63.93 | 5.40 | 4.12 | 5.88 | 7.84 | 9.44 |
| T₅ – 48 hours soaking in 25% cow urine | 8.16 | 17.33 | 72.16 | 7.05 | 4.90 | 7.27 | 9.47 | 11.49 |
| T₆ – 48 hours soaking in 50% cow urine | 7.83 | 18.00 | 71.38 | 6.85 | 4.75 | 6.77 | 9.58 | 10.65 |
| T₇ – 48 hours soaking in 100% cow urine | 8.16 | 18.83 | 70.73 | 6.48 | 4.65 | 6.71 | 8.82 | 10.61 |
| S.E. | 0.15 | 0.20 | 0.24 | 0.02 | 0.01 | 0.040 | 0.056 | 0.091 |
| CD at 5% level | 0.44 | 0.59 | 0.71 | 0.07 | 0.05 | 0.115 | 0.162 | 0.263 |

Table 2: Influence of seed soaking duration and concentration of cow urine on growth parameters at different intervals

| Treatments | Stem girth (mm) | Number of leaves per seedling | Length of seedling at 120 DAS (cm) | Number of roots per seedling at 120 DAS | Fresh weight of shoots at 120 DAS (g) | Dry weight of shoots at 120 DAS (g) | Fresh weight of roots at 120 DAS (g) | Dry weight of roots at 120 DAS (g) |
|------------|----------------|-----------------------------|-----------------------------------|----------------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|
| T₀ - control (tap water) | 1.52 | 1.88 | 2.13 | 16.20 | 36.20 | 49.86 | 19.40 | 43.93 | 1.23 | 0.41 | 0.37 | 0.14 |
| T₁ – 24 hours soaking in 25% cow urine | 1.93 | 2.27 | 2.56 | 28.50 | 66.76 | 81.76 | 27.96 | 105.71 | 2.50 | 1.05 | 0.74 | 0.33 |
| T₂ – 24 hours soaking in 50% cow urine | 1.87 | 2.18 | 2.44 | 26.90 | 63.63 | 73.50 | 26.17 | 101.50 | 2.33 | 0.83 | 0.67 | 0.23 |
| T₃ – 24 hours soaking in 100% cow urine | 1.84 | 2.18 | 2.41 | 23.06 | 60.06 | 70.13 | 24.32 | 87.80 | 2.23 | 0.75 | 0.55 | 0.22 |
| T₄ – 48 hours soaking in tap water | 1.55 | 1.91 | 2.14 | 16.20 | 36.60 | 50.43 | 19.99 | 43.10 | 1.20 | 0.38 | 0.36 | 0.15 |
| T₅ – 48 hours soaking in 25% cow urine | 1.78 | 2.16 | 2.36 | 22.56 | 51.86 | 65.16 | 22.32 | 83.08 | 2.11 | 0.72 | 0.54 | 0.21 |
Table 3: Influence of seed soaking duration and concentration of cow urine on growth and physiological parameters at 120 days after sowing

| Treatments | Seedling vigor Index I (cm) | Seedling vigor index II (g) | Survival of seedlings (%) | Leaf Area Index | Leaf Area Duration (cm².days) | Light Transmission Ratio (%) | Energy Interception (cal cm⁻² min⁻¹) |
|------------|-----------------------------|-----------------------------|---------------------------|----------------|-----------------------------|-----------------------------|---------------------------------|
| T₀ - control (tap water) | 1210.91 | 36.57 | 64.71 | 1.25 | 1904.31 | 76.30 | 0.12 |
| T₁ - 24 hours soaking in 25% cow urine | 2190.44 | 101.63 | 78.78 | 3.75 | 6181.45 | 36.28 | 0.40 |
| T₂ - 24 hours soaking in 50% cow urine | 1995.13 | 81.22 | 77.78 | 2.75 | 4553.71 | 44.35 | 0.34 |
| T₃ - 24 hours soaking in 100% cow urine | 1730.63 | 69.38 | 72.00 | 2.46 | 4075.26 | 45.31 | 0.22 |
| T₄ - 48 hours soaking in water | 1246.19 | 33.73 | 64.85 | 1.30 | 1929.26 | 76.00 | 0.12 |
| T₅ - 48 hours soaking in 25% cow urine | 1554.43 | 65.82 | 71.53 | 2.23 | 3632.71 | 47.19 | 0.21 |
| T₆ - 48 hours soaking in 50% cow urine | 1473.01 | 60.51 | 70.55 | 2.07 | 3332.45 | 53.89 | 0.19 |
| T₇ - 48 hours soaking in 100% cow urine | 1457.74 | 59.47 | 69.38 | 1.96 | 3066.08 | 55.62 | 0.18 |
| SE± | 21.71 | 0.88 | 0.37 | 0.02 | 41.50 | 0.40 | 0.003 |
| CD at 5% level | 62.73 | 2.54 | 1.07 | 0.06 | 119.91 | 1.18 | 0.009 |

Conclusion
Salient findings from the present study revealed the efficacy of soaking seeds in 25% cow urine for 24 hours supplements in improving seed germination, growth and survival of Karonda seeds. This combination achieved high seed vigor and physiological growth of the seedlings, Sankaranarayana et al. (1994) [6] reported that germination to be accelerated by soaking of tamarind seeds in 10 per cent cow urine for 24 hours.

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