Effect of Different Pre Sowing Treatments on Seed Germination and Growth of Custard Apple (Annona squamosa L.) Seedlings

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A B S T R A C T

The result of the investigation revealed that there were significant variations in germination and seedling growth of custard apple due to cow urine concentrations with soaked duration and PGPR inoculation and interaction of three factors. Amongst the different cow urine concentration G₁ (20%) recorded earliest germination (22.3 days), 50% germination (40.2 days) % of germination at 30 days (43.5 %), percent of germination at 60 days (70.4 %), whereas S₁ contributed to minimum days (22.3) to taken start germination, 50% germination (43.6 days) and % of germination at 30 days (43.5 %). At 60 days (61.3 %). Growing media (PGPR), Contribute to early germination days taken to start germination, days taken to 50% germination, % of germination at 30 days % of germination at 60 days were better in 6% growing media under P₃. Among the three factors cow urine was superior in respect to germination parameters. The height of shoot (5.5, 7.8, 12.2, 13.3 cm), no. of leaves (5.7, 6.6, 9.5, 11.0), girth of stem (1.6, 2.2, 2.5, 3.0) under G₁. Maximum height of shoot (5.7, 8.6, 12.6, 14.5) was recorded among the three factors interaction G₁S₁P₃. The seedling height (22.8 cm), no. of roots (32.0), fresh weight of shoot and dry weight of shoot (2.1 g and 0.64 g), fresh weight of root and dry weight of root (0.64 and 0.48 g), were maximum under (G₁) 20 % cow urine. Speed of germination (0.68 seed/ day), coefficient velocity of germination (2.61) were maximum under G₁P₃ treatment combination. The leaf area index during 120-150 days (3.9), leaf area duration (1508.84 cm² days), energy interception (0.49) recorded under G₁ (20% Cow urine). The maximum LTR was recorded under G₀ (control). Seeding vigour index I (1075.66 cm), Seedling vigour II (58.83 G), % of survival (58%) was recorded and it was maximum under 20 % cow urine. While soaked duration contributed to (1072 cm), (50.16 g) under S₁ (24hrs) soaked duration. The interaction between cow urine and soaked duration was superior among all the interaction height of shoot at various stages (4.4, 7.1, 12,6,13.9 cm), no. of leaves (5.5, 6.7, 9.2, 10.8), girth of stem (1.4, 2.1, 2.0, 2.7) Speed of germination, coefficient velocity of germination, were recorded under G₁S₁ treatment combination. Combine effect of cow urine and PGPR recorded no. of roots (28), height of seedling (21.24 cm), length of root (9.2 cm), days taken to start germination (22.41), % of germination at 30 and 60 days (51% and 83.3 %) under G₁P₃, it was superior over rest to cow urine with growing media treatments. Interaction among three factors was non-significant the parameters, days taken to 50% germination, fresh and dry weight of shoot, fresh weight of shoot, leaf area duration, leaf transmission ratio, speed of germination, coefficient velocity of germination energy interception.

Keywords
Seed germination, Pre sowing treatments, Custard apple

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Introduction

Custard apple (*Annona squamosa* L.) belongs to family Annonaceae. The family annonaceae belongs to suborder Mangolineae, Myristicaceae and lauraceae. It is one of the finest fruit gifted to India by tropical America. It has several synonyms such as SitafalSharifa, Sugar apple, Sweet sop etc., Custard apple is generally used as fresh pulp of fruit is juicy, cream, yellow, or white, sweet with pleasant texture and flavour. It contains 28.6-36.9% edible portion consisting of 12.4-16.6% sugar, 73.30 moisture,1.60 % protein, 0.30% fat, 0.70% mineral matter,23.90% carbohydrate, 0.20% calcium, 0.40 % phosphorus,1.0% iron and 0.26-0.65% acidity with caloric value of 105 K cal /100g besides high nutritive value, it has also a high medicinal value un.

The fruits are often scaly and succulent and are sometimes segmented. Annonas are mostly grown by seeds either for seedling plants or for vegetative propagation but it can also be propagate by inarching, budding and grafting. There are not common cultivars of this fruit, but it can be classified according to their yellowish and brownish skin colours. The tree is fast growing in nature so, responded positive to the cultural practices i.e. mulching, organic fertilizers, frequent irrigation etc. The fruiting of plant starts after two to three years, plant hold fruit two to three months for repening process. The seeds of Annonaceae are albuminous ellipsoids and their length varies between 5 and 30 mm. ripe fruits, seeds, leaf and root are considered as medicines.

Seed germination is the first stage of plant development, it is the resumption of active growth of embryo that results in the emergence of the young plant. Dormancy in seeds may be due to presence of hard and impermeable seed coat, germination inhibitors and improper development of embryo. To get higher and proper germination seed needs pre sowing treatments which helps in promotion of early and higher percentage of seed germination with healthy vigorous seedling. Seed germination is affected by many factors, which include type of substrate used, environmental factors such as oxygen, water, temperature, and light. Seed without use of growth regulators showed poor response to germination and growth. In recent year, lots of research work has been done on different aspects of growth regulators. The important aspects among them are the most appropriate growth regulators for encouraging germination and further growth. The prices of the growth regulators have sky high so the nursery man who is the regular consumer can’t afford it much longer. To overcome this crisis some alternative for growth regulators should be brought up which may be as efficient as growth regulators. Easy to access and cheap. This has diverted the attention once again towards Can sugar, cow urine, bio-inoculants (Rhizobacteria).

Cow urine proving feasible may bring a breakthrough in the present context as it is free of cost and easily available through it is not much consistent. Cow urine contains Nitrogen, Sulphur Ammonia, Copper, Iron, Urea, Uric Acid, Phosphorus, Sodium, Potassium, Manganese, Carbolic Calcium, Salt, Vitamins, Lactose, Enzyme, Water, Creatin, Aurum Hydroxide etc. (Dilrukshi, 2009). The enough work has been done on seed germination with use of PGR’s and growing media but there is meager work on seed treatment with organic matter like cow urine with soaking period and incorporation of plant growth promoting Rhizobacteria in growing media. In this context, the utilization of plant growth promoting Rhizobacteria (PGPR) may help to develop an environmentally benign biological approach for managing fungal diseases and enhancing the plant health resulting increase the yield.
Materials and Methods

The experiment was conducted at vegetable Research Farm, Maharajpur, Department of Horticulture, JNKVV, Jabalpur (M.P.) during 2018-2019. Jabalpur is situated in “Kymore Plateau and Satpura Hills” Agro-climatic zone of Madhya Pradesh at 23.9°North latitude and 79.58°east longitudes and an altitude of 411.78 meter above the mean sea level. In the experiment the response of different concentration of cow urine, soaking duration and growing media (PGPR) were studied for seed germination, growth and survival of Custard apple seedlings. The experiment was laid out in Asymmetrical Factorial Randomized Block Design with 54 treatment combinations replicated thrice, Observations were recorded using standard procedure and statistically analysed (Table 1).

\[
\text{Total no. of seeds germinated} \quad \text{Germination (\%)} = \frac{\text{Total no of seeds sown}}{100} \\
\]

\[
\text{CVG} = \sum N_i + \sum N_i X \sum T_i + \sum N_i T_i \\
\]

\[
\text{Total Leaf Area} = \text{LAI} = \frac{\text{Total Leaf Area}}{\text{Ground area}} \\
\]

Results and Discussion

Growth parameter

The following observations were recorded at different time intervals after sowing. Randomly selected five plants were tagged for following observations.

% of germination at 30 and 60 DAS

Data pertaining germination at 30 and 60 days has given in table 2 and Data revealed that cow urine had showed significant effect on percent of germination. The maximum germination percent of 44.8 and 70.4 was recorded under cow urine concentration (20 %) G1 at 30 and 60 days after sowing, respectively. minimum germination percent of 39.3 and 56.7 was recorded under seed soaked in tap water (G0) after sowing. It is apparent from analysed data that soaking duration significantly affected the percent of germination. The maximum germination percent 43.1 and 61.3 was recorded under S1 (seed soaked for 24 hrs) while, minimum germination percent of 40.8 and 58.1 was recorded under S3 (seed soaked for 72 hrs).The growing media in rich with PGPR had showed the significant effect on germination. The maximum germination percentage of 42.6 and 62.5 was recorded under P3 (6%). Whereas, minimum germination of 41.6 and 60.4 was recorded under P1 at 30 and 60 days after sowing. Concentration of cow urine and soaking duration had showed significant effect on germination and maximum germination percent of 47.0 and 75.3 was recorded under seed soaked in 20 % cow urine for 24 hours (G1S1) while, minimum Germination percentage of 38.5 and 55.5 was recorded under G0S3 at 30 and 60 days after seed sowing, respectively.

Combine effect of cow urine and growing media (PGPR) had showed significant effect on germination and the maximum germination percentage of 45.5 and 75.3 was recorded at 30 and 60 days after sowing, respectively under G1P3. While minimum germination percent of 39.1 and 56.2 was recorded under G0P1 at 30 and 60 days after sowing, respectively.

Coefficient velocity of germination

The data presented in table 3 indicated that the cow urine and growing media had showed significant effect on coefficient velocity of germination. Whereas, soaking duration had
not shown any significant effect on coefficient velocity of germination. The maximum coefficient velocity of 2.7 was observed under seed soaked in cow urine concentration (20%) and minimum 2.1 seed/day under tap water. As regards soaking duration had showed non-significant effect on coefficient velocity of germination. As regards PGPR in rich with growing media showed significant effect on coefficient velocity of germination. The maximum coefficient velocity of germination 2.4 was noted under P3 and whereas, minimum value of 2.3 was noted under control (P1). As regards interaction of cow urine and soaking duration had showed non-significant effect on coefficient velocity of germination. Interaction of cow urine and growing media showed non-significant effect on coefficient velocity of germination. The maximum coefficient velocity of germination 2.7 was noted under G1P3 and whereas, minimum value of 2.3 was noted under G0P1. Interaction of cow urine, soaking duration and growing media showed non-significant effect on CVG while the maximum CVG was recorded under G1S1P3.

**Height of shoot at 60, 90, 120 and 150 days**

The height of shoots at 60, 90, 120, 150 days after sowing was recorded and presented in Table. Data showed that the cow urine showed significant effect on height of shoots at all the stages. The maximum shoot height of 5.0, 7.3, 12.1 and 13.3 cm was noted under cow urine concentration of (20%). minimum shoot height was recorded under (G0) seed soaked in tap water at 60, 90, 120 and 150 days after seed sowing respectively. Cow urine soaking concentration (20%) showed superiority over rest of the concentrations. As regards to soaking duration had showed significant effect on height of shoot and maximum height of 5.0, 6.8, 11.1 and 13.4 cm was noted under S1 i.e. seed soaked for 24 hrs of soaked duration at 60, 90, 120 and 150 days after seed sowing, respectively and found significantly superior over rest of the duration of seed soaking duration whereas, the minimum height of shoots 4.0, 6.2, 9.8 and 12.0 cm was recorded under S3 at 60, 90, 120 and 150 days after seed sowing, respectively.

Inoculation with PGPR in growing media had showed significant effect on height of shoot at various stages. The maximum height of 4.7, 6.6, 10.8, 12.9 was noted under seed grown in PGPR rich (6%) growing media at 60,90,120 and 150 days after sowing, respectively whereas, the minimum value noted under control (P1). Interaction effect of cow urine x soaking duration had showed significant effect and maximum shoot height recorded under G1S1 (5.2, 7.8,12.6 and 13.9 cm) whereas, the minimum shoot height was 3.5, 5.9, 6.10.5 cm was recorded under seed soaked for 72 hours in tap water at 60,90,120 and 150 days after sowing. As regards the cow urine and growing media had showed significant effect on height of shoot and the maximum shoot height was of 5.4, 7.5, 12.3 and 13.6 cm under G1P3 whereas, minimum shoot height of 3.8, 6.0, 8.9 and 10.9 cm under G0P1 respectively at 60,90,120 and 150 days after sowing.

Interaction effect of soaking duration and growing media had significant effect on height of shoot at various stages and maximum shoot height noted under S1P3 5.7, 7.0, 12.2 and 13.8 cm was recorded under seed soaked for 24 hrs and growing media in rich with 6% PGPR. Whereas, the minimum shoot height was observed under S3P1. Combine effect of cow urine, soaking duration and growing media had showed significant effect on shoot height at various stages and the maximum shoot height was recorded under treatment combination of cow urine concentration (20%), soaking duration
(24 hrs), and growing media in rich with PGPR (6%).

**Length of seedlings at 150 days after sowing**

The significantly maximum height of seedling (24.4 cm) at 150 DAS was recorded under the treatment $G_1$. The present investigations are in conformity with the results of Shristava and Bhel (2002). Probable reasons may be that the cow urine which induced the cell elongation process and ultimately increased the height of the plant. These results are supported by Rao (1975) and Parmar *et al.*, The maximum plant growth (23.84) in PGPR was possibly due to some plant growth promoting rhizobacteria (pseudomonas) that is increase physiological activities of seed, essential for cell division and cell enlargement. Krishnamoothy and Vajranrabhaian (1986) who reported that some plant growth promoting rhizobacteria releases such as cytokinins and auxins like substances. It also contains rich source of nutrient. The results obtained were closely related with the finding of Kumar and Sharma 2007 in *Jatropha curcas*. As regard to cow urine+ soaking duration + PGPR interaction combinedly affected a significant increase in length of seedlings (29.8 cm) at 150 days after sowing was observed. Due to inter-nodal cell elongation, there by leading to increase in seedling length. These findings are supported by Ratan and Reddy (2004); Parmar *et al.*, 2016 (Table 5).

**Seedling vigour index I and seedling vigour index II**

The data reveals the significant effect of organic sources on seedling vigour index I and seedling vigour index II. The seedling vigour index I (1090.74 cm and 69.8 g) was recorded under $G_1S_1P_3$ which is superior to all other combination of cow urine. It is followed by cow urine + soaked duration/cow urine + PGPR concentration. The findings are supported by Gurung *et al.*, (2014).

**Energy interception**

In the present study, all the treatment growing media, gibberellic acid concentration and their combination effect showed significant effect on the experiment concern. The probable reason may be that Interception of light by a crop canopy is strongly related to total leaf area. A crop will thus intercept more PAR and hence grow faster if it develops leaf area rapidly. Similar findings were reported by Maddonni and Otegui (1996) (Table 4 and 6).

| Factor A                | Factor B (Soaked Time) | Factor C (PGPR) |
|-------------------------|------------------------|-----------------|
| $G_0$ Tap Water-100ml   | $S_1$ 24 hours         | $P_1$ 0%        |
| $G_1$ Cow Urine-20%     | $S_2$ 48 hours         | $P_2$ 3%        |
| $G_2$ Cow Urine-40%     | $S_3$ 72 hours         | $P_3$ 6%        |
| $G_3$ Cow Urine-60%     |                        |                 |
| $G_4$ Cow Urine-80%     |                        |                 |
| $G_5$ Cow Urine-100%    |                        |                 |
Table 2 Effect of seed soaking duration, concentration of cow urine and growing media on germination at 30 and 60 DAS (%)

| Treatment                | Growing media | Mean |
|--------------------------|---------------|------|
|                          | Without PGPR (P₁) | With PGPR (P₂) | With PGPR (P₃) |      |
| Soaking duration -24 hr (S₁) | 60.7          | 61.3  | 62.0  | 61.3 |
| Soaking duration -48 hr (S₂) | 59.3          | 59.6  | 60.4  | 59.8 |
| Soaking duration -72 hr (S₃) | 58.7          | 56.8  | 58.9  | 58.1 |
| Mean                     | 60.0          | 60.1  | 61.4  |      |

Growing media (P) Soaking duration (S) S x P

SEm± 0.079 0.079 0.136
CD at 5 % 0.221 0.221 0.382

| Growing media                       | Without PGPR (P₁) | With PGPR (P₂) | With PGPR (P₃) | Mean |
|-------------------------------------|-------------------|---------------|---------------|------|
| Tape water (G₀)                     | 56.2              | 56.8          | 57.2          | 56.7 |
| Cow urine Conc.- 20% (G₁)          | 61.6              | 62.6          | 75.3          | 70.4 |
| Cow urine Conc.- 40% (G₂)          | 61.3              | 62.2          | 62.1          | 62.4 |
| Cow urine Conc.- 60% (G₃)          | 59.6              | 60.0          | 60.2          | 60.8 |
| Cow urine Conc.- 80% (G₄)          | 58.3              | 59.3          | 59.2          | 59.2 |
| Cow urine Conc.- 100% (G₅)        | 56.2              | 57.5          | 58.2          | 57.5 |
| Mean                               | 60.0              | 60.1          | 61.4          |      |

Growing media (P) Concentration (G) G x P

SEm± 0.079 0.111 0.193
CD at 5 % 0.221 0.312 0.558

| Soaking Duration | 24 hr (S₁) | 48 hr (S₂) | 72 hr (S₃) | Mean |
|------------------|------------|------------|------------|------|
| Tape water (G₀)  | 58.2       | 56.5       | 55.5       | 56.7 |
| Cow urine Conc.- 20% (G₁) | 75.3    | 64.1       | 62.4       | 70.4 |
| Cow urine Conc.- 40% (G₂) | 64.1    | 63.2       | 60.8       | 62.4 |
| Cow urine Conc.- 60% (G₃) | 61.6    | 59.5       | 58.5       | 60.8 |
| Cow urine Conc.- 80% (G₄) | 60.7    | 59.6       | 57.4       | 59.2 |
| Cow urine Conc.- 100% (G₅) | 59.0    | 57.2       | 56.3       | 57.5 |
| Mean              | 61.3       | 59.8       | 58.1       |      |

Duration (S) Concentration (G) G x S

SEm± 0.079 0.111 0.193
CD at 5 % 0.221 0.312 0.540
## Treatment

| Soaking duration | Growing media | Mean |
|------------------|----------------|------|
| 24 hr (S<sub>1</sub>) | Without PGPR (P<sub>1</sub>) | 43.1 |
|                   | With PGPR (P<sub>2</sub>) | 43.3 |
|                   | With PGPR (P<sub>3</sub>) | 44.2 |
| 48 hr (S<sub>2</sub>) | Without PGPR (P<sub>1</sub>) | 41.5 |
|                   | With PGPR (P<sub>2</sub>) | 41.8 |
|                   | With PGPR (P<sub>3</sub>) | 42.3 |
| 72 hr (S<sub>3</sub>) | Without PGPR (P<sub>1</sub>) | 40.3 |
|                   | With PGPR (P<sub>2</sub>) | 40.7 |
|                   | With PGPR (P<sub>3</sub>) | 41.3 |

**Growing media (P)**

**Soaking duration (S)**

**S x P**

| SE± | 0.091 | 0.091 | 0.157 |
|---|---|---|---|
| CD at 5 % | 0.254 | 0.254 | 0.471 |

### Growing media

| Growing media | Without PGPR (P<sub>1</sub>) | With PGPR (P<sub>2</sub>) | With PGPR (P<sub>3</sub>) | Mean |
|---------------|-------------------------------|--------------------------|--------------------------|------|
| Tape water (G<sub>0</sub>) | 39.1                          | 39.3                     | 39.6                     | 39.3 |
| Cow urine Conc.- 20% (G<sub>1</sub>) | 44.4                          | 44.5                     | 45.5                     | 44.8 |
| Cow urine Conc.- 40% (G<sub>2</sub>) | 43.0                          | 43.5                     | 44.0                     | 43.1 |
| Cow urine Conc.- 60% (G<sub>3</sub>) | 42.2                          | 42.5                     | 43.5                     | 42.7 |
| Cow urine Conc.- 80% (G<sub>4</sub>) | 41.0                          | 41.1                     | 42.0                     | 41.4 |
| Cow urine Conc.- 100% (G<sub>5</sub>) | 39.8                          | 40.3                     | 41.1                     | 40.4 |

**Growing media (P)**

**Concentration (G)**

**G x P**

| SE± | 0.091 | 0.128 | 0.222 |
|---|---|---|---|
| CD at 5 % | 0.254 | 0.359 | 0.622 |

### Soaking Duration

| Soaking Duration | 24 hr (S<sub>1</sub>) | 48 hr (S<sub>2</sub>) | 72 hr (S<sub>3</sub>) | Mean |
|------------------|-----------------------|-----------------------|-----------------------|------|
| Tape water (G<sub>0</sub>) | 40.3                  | 39.2                  | 38.5                  | 39.3 |
| Cow urine Conc.- 20% (G<sub>1</sub>) | 47.0                  | 44.3                  | 44.2                  | 44.8 |
| Cow urine Conc.- 40% (G<sub>2</sub>) | 44.7                  | 43.3                  | 42.4                  | 43.1 |
| Cow urine Conc.- 60% (G<sub>3</sub>) | 44.7                  | 42.4                  | 41.4                  | 42.7 |
| Cow urine Conc.- 80% (G<sub>4</sub>) | 42.5                  | 41.4                  | 41.0                  | 41.4 |
| Cow urine Conc.- 100% (G<sub>5</sub>) | 41.6                  | 40.3                  | 39.3                  | 40.4 |

**Soaking duration (G)**

**Duration (S)**

**G x S**

| SE± | 0.091 | 0.128 | 0.222 |
|---|---|---|---|
| CD at 5 % | 0.254 | 0.359 | 0.622 |
Table 3 Effect of seed soaking duration, concentration of cow urine and growing media on Coefficient velocity of germination

| Treatment                  | Growing media | Mean |
|----------------------------|---------------|------|
|                            | Without PGPR (P<sub>1</sub>) | With PGPR (P<sub>2</sub>) | With PGPR (P<sub>3</sub>) |
| Soaking duration -24 hr (S<sub>1</sub>) | 2.2           | 2.3  | 2.4  | 2.3  |
| Soaking duration -48 hr (S<sub>2</sub>) | 2.3           | 2.3  | 2.3  | 2.3  |
| Soaking duration -72 hr (S<sub>3</sub>) | 2.2           | 2.3  | 2.3  | 2.2  |
| Mean                       | 2.3           | 2.3  | 2.4  |

Growing media (P) Soaking duration (S) S x P
SEm± 0.012 0.012 0.021
CD at 5 % NS NS

### Growing media

| Growing media                  | Without PGPR (P<sub>1</sub>) | With PGPR (P<sub>2</sub>) | With PGPR (P<sub>3</sub>) | Mean |
|-------------------------------|-------------------------------|----------------------------|----------------------------|------|
| Tape water (G<sub>0</sub>)    | 2.1                           | 2.2                       | 2.2                       | 2.1  |
| Cow urine Conc.- 20% (G<sub>1</sub>) | 2.5                       | 2.7                       | 2.7                       | 2.7  |
| Cow urine Conc.- 40% (G<sub>2</sub>) | 2.3                       | 2.4                       | 2.5                       | 2.4  |
| Cow urine Conc.- 60% (G<sub>3</sub>) | 2.3                       | 2.4                       | 2.3                       | 2.4  |
| Cow urine Conc.- 80% (G<sub>4</sub>) | 2.2                       | 2.3                       | 2.3                       | 2.2  |
| Cow urine Conc.- 100% (G<sub>5</sub>) | 2.1                       | 2.2                       | 2.1                       | 2.1  |
| Mean                          | 2.3                           | 2.3                       | 2.4                       |

Growing media (P) Concentration (G) G x P
SEm± 0.012 0.017 0.030
CD at 5 % 0.032 0.049 0.084

### Soaking Duration

| Soaking Duration | 24 hr (S<sub>1</sub>) | 48 hr (S<sub>2</sub>) | 72 hr (S<sub>3</sub>) | Mean |
|------------------|------------------------|------------------------|------------------------|------|
| Tape water (G<sub>0</sub>) | 2.2                    | 2.1                    | 2.1                    | 2.1  |
| Cow urine Conc.- 20% (G<sub>1</sub>) | 2.7                    | 2.7                    | 2.6                    | 2.7  |
| Cow urine Conc.- 40% (G<sub>2</sub>) | 2.5                    | 2.4                    | 2.3                    | 2.4  |
| Cow urine Conc.- 60% (G<sub>3</sub>) | 2.4                    | 2.3                    | 2.3                    | 2.4  |
| Cow urine Conc.- 80% (G<sub>4</sub>) | 2.3                    | 2.2                    | 2.2                    | 2.2  |
| Cow urine Conc.- 100% (G<sub>5</sub>) | 2.1                    | 2.1                    | 2.1                    | 2.1  |
| Mean             | 2.3                    | 2.3                    | 2.2                    |

Duration (S) Concentration (G) G x S
SEm± 0.012 0.017 0.030
CD at 5 % 0.049 0.084
Table 4: Effect of cow urine, soaking duration and growing media on EI during 120-150 DAS

| Treatment                   | Growing media                                                                 | Mean  |
|-----------------------------|-----------------------------------------------------------------------------|-------|
|                             | Without PGPR (P<sub>1</sub>)       | With PGPR (P<sub>2</sub>) | With PGPR (P<sub>3</sub>) |
| Soaking duration -24 hr (S<sub>1</sub>) | 0.46                           | 0.48       | 0.49       | 0.48       |
| Soaking duration -48 hr (S<sub>2</sub>) | 0.43                           | 0.44       | 0.45       | 0.44       |
| Soaking duration -72 hr (S<sub>3</sub>) | 0.40                           | 0.41       | 0.43       | 0.41       |
| Mean                        | 0.43                           | 0.44       | 0.46       |            |
| Growing media (P)           | Soaking duration (S)            | S x P      |            |            |
| SEm±                        | 0.001                          | 0.001      | 0.007      | 0.002      |
| CD at 5 %                   | 0.003                          | 0.003      | 0.007      | 0.007      |

| Treatment                   | Growing media                                                                 | Mean  |
|-----------------------------|-----------------------------------------------------------------------------|-------|
|                             | Without PGPR (P<sub>1</sub>)       | With PGPR (P<sub>2</sub>) | With PGPR (P<sub>3</sub>) |
| Tape water (G<sub>0</sub>)  | 0.39                           | 0.40       | 0.41       | 0.40       |
| Cow urine Conc.- 20% (G<sub>1</sub>) | 0.48                         | 0.49       | 0.50       | 0.49       |
| Cow urine Conc.- 40% (G<sub>2</sub>) | 0.46                         | 0.47       | 0.49       | 0.47       |
| Cow urine Conc.- 60% (G<sub>3</sub>) | 0.44                         | 0.46       | 0.48       | 0.46       |
| Cow urine Conc.- 80% (G<sub>4</sub>) | 0.43                         | 0.43       | 0.45       | 0.44       |
| Cow urine Conc.- 100% (G<sub>5</sub>) | 0.40                         | 0.41       | 0.41       | 0.41       |
| Mean                        | 0.43                           | 0.44       | 0.46       |            |
| Growing media (P)           | Concentration (G)              | G x P      |            |            |
| SEm±                        | 0.001                          | 0.001      | 0.002      | 0.002      |
| CD at 5 %                   | 0.003                          | 0.003      | 0.007      | 0.007      |

| Treatment                   | Soaking Duration          | Mean  |
|-----------------------------|---------------------------|-------|
|                             | 24 hr (S<sub>1</sub>)     | 48 hr (S<sub>2</sub>) | 72 hr (S<sub>3</sub>) |
| Tape water (G<sub>0</sub>)  | 0.42                       | 0.40       | 0.38       | 0.40       |
| Cow urine Conc.- 20% (G<sub>1</sub>) | 0.54                   | 0.49       | 0.44       | 0.49       |
| Cow urine Conc.- 40% (G<sub>2</sub>) | 0.52                   | 0.47       | 0.43       | 0.47       |
| Cow urine Conc.- 60% (G<sub>3</sub>) | 0.50                   | 0.46       | 0.41       | 0.46       |
| Cow urine Conc.- 80% (G<sub>4</sub>) | 0.46                   | 0.44       | 0.41       | 0.44       |
| Cow urine Conc.- 100% (G<sub>5</sub>) | 0.43                   | 0.40       | 0.39       | 0.41       |
| Mean                        | 0.48                       | 0.44       | 0.41       |            |
| Duration (S)                | Concentration (G)          | G x S      |            |            |
| SEm±                        | 0.001                      | 0.001      | 0.002      | 0.002      |
| CD at 5 %                   | 0.003                      | 0.003      | 0.007      | 0.007      |
**Table 5** Effect of cow urine, soaking duration and growing media on seedling length after sowing 150 days

| Treatment          | Growing media         |                |                |                | Mean   |
|--------------------|-----------------------|----------------|----------------|----------------|--------|
|                    | Without PGPR (P₁)     | With PGPR (P₂) | With PGPR (P₃) |                |        |
| Soaking duration   |                       |                |                |                |        |
| - 24 hr (S₁)      | 23.3                  | 23.3           | 24.2           | 23.8           |        |
| - 48 hr (S₂)      | 21.7                  | 22.1           | 22.3           | 22.0           |        |
| - 72 hr (S₃)      | 21.1                  | 21.2           | 21.3           | 21.2           |        |
| Mean               | 22.1                  | 22.4           | 22.6           |                |        |
| Growing media (P) | Soaking duration (S)  | S x P          |                |                |        |
| SEM±               | 0.355                 | 0.355          | 0.614          |                |        |
| CD at 5 %          | 0.994                 | 0.994          | 1.839          |                |        |

| Growing media     | Without PGPR (P₁)     | With PGPR (P₂) | With PGPR (P₃) | Mean   |
|--------------------|-----------------------|----------------|----------------|--------|
| Tape water (G₀)    | 20.1                  | 20.2           | 20.5           | 20.3   |
| Cow urine Conc.- 20% (G₁) | 24.1              | 24.5           | 24.6           | 24.4   |
| Cow urine Conc.- 40% (G₂) | 22.5              | 22.8           | 23.1           | 22.8   |
| Cow urine Conc.- 60% (G₃) | 22.2              | 22.7           | 22.9           | 22.6   |
| Cow urine Conc.- 80% (G₄) | 22.1              | 22.4           | 22.7           | 22.4   |
| Cow urine Conc.- 100% (G₅) | 21.3             | 21.8           | 21.          | 21.6   |
| Mean               | 22.1                  | 22.4           | 22.6           |        |
| Growing media (P) | Concentration (G)     | G x P          |                |        |
| SEM±               | 0.355                 | 0.501          | 0.868          |        |
| CD at 5 %          | 0.994                 | 1.406          | 2.260          |        |

| Soaking Duration  | 24 hr (S₁) | 48 hr (S₂) | 72 hr (S₃) | Mean   |
|--------------------|------------|------------|------------|--------|
| Tape water (G₀)    | 20.9       | 20.5       | 19.4       | 20.3   |
| Cow urine Conc.- 20% (G₁) | 29.8       | 22.1       | 21.4       | 24.4   |
| Cow urine Conc.- 40% (G₂) | 23.5       | 22.6       | 22.3       | 22.8   |
| Cow urine Conc.- 60% (G₃) | 23.2       | 22.5       | 22.1       | 22.6   |
| Cow urine Conc.- 80% (G₄) | 23.1       | 22.4       | 21.6       | 22.4   |
| Cow urine Conc.- 100% (G₅) | 22.6       | 21.7       | 20.6       | 21.6   |
| Mean               | 23.8       | 22.0       | 21.2       |        |
| Duration (S)       | Concentration (G)   | G x S        |            |        |
| SEM±               | 0.355       | 0.501      | 0868       |        |
| CD at 5 %          | 0.994       | 1.406      | 2.435      |        |

Cow urine x soaking duration x growing media

| SEM±               | 1.504       |        |        |        |
| CD at 5 %          | 4.334       |        |        |        |
Table 6: Effect of cow urine, soaking duration and growing media on LAI at 120-150

| Treatment                               | Growing media Without PGPR (P<sub>1</sub>) | With PGPR (P<sub>2</sub>) | With PGPR (P<sub>3</sub>) | Mean |
|-----------------------------------------|--------------------------------------------|---------------------------|---------------------------|------|
| Soaking duration -24 hr (S<sub>1</sub>) | 3.7                                        | 3.8                       | 3.8                       | 3.8  |
| Soaking duration -48 hr (S<sub>2</sub>) | 3.7                                        | 3.7                       | 3.7                       | 3.7  |
| Soaking duration -72 hr (S<sub>3</sub>) | 3.6                                        | 3.7                       | 3.7                       | 3.7  |
| Mean                                    | 3.7                                        | 3.7                       | 3.8                       |      |

| Growing media (P) | Soaking duration (S) | S x P | SE±m | CD at 5 % |
|-------------------|----------------------|-------|------|-----------|
|                   |                      |       | 0.011| 0.033     |
|                   |                      |       | 0.011| 0.030     |
|                   |                      |       | 0.018| 0.054     |

| Growing media | Without PGPR (P<sub>1</sub>) | With PGPR (P<sub>2</sub>) | With PGPR (P<sub>3</sub>) | Mean |
|---------------|--------------------------------|---------------------------|---------------------------|------|
| Tape water (G<sub>0</sub>) | 3.4                            | 3.5                       | 3.5                       | 3.5  |
| Cow urine Conc.- 20% (G<sub>1</sub>) | 3.9                            | 4.0                       | 4.1                       | 4.0  |
| Cow urine Conc.- 40% (G<sub>2</sub>) | 3.9                            | 3.9                       | 3.9                       | 3.9  |
| Cow urine Conc.- 60% (G<sub>3</sub>) | 3.8                            | 3.9                       | 3.9                       | 3.9  |
| Cow urine Conc.- 80% (G<sub>4</sub>) | 3.7                            | 3.8                       | 3.8                       | 3.7  |
| Cow urine Conc.- 100% (G<sub>5</sub>) | 3.6                            | 3.6                       | 3.6                       | 3.5  |
| Mean          | 3.7                            | 3.7                       | 3.8                       |      |

| Growing media (P) | Concentration (G) | G x P | SE±m | CD at 5 % |
|-------------------|-------------------|-------|------|-----------|
|                   |                   |       | 0.011| 0.033     |
|                   |                   |       | 0.015| 0.042     |
|                   |                   |       | 0.026| 0.078     |

| Soaking Duration | 24 hr (S<sub>1</sub>) | 48 hr (S<sub>2</sub>) | 72 hr (S<sub>3</sub>) | Mean |
|------------------|------------------------|------------------------|------------------------|------|
| Tape water (G<sub>0</sub>) | 3.5                    | 3.5                    | 3.4                    | 3.5  |
| Cow urine Conc.- 20% (G<sub>1</sub>) | 4.0                    | 3.9                    | 3.9                    | 4.0  |
| Cow urine Conc.- 40% (G<sub>2</sub>) | 3.9                    | 3.9                    | 3.8                    | 3.9  |
| Cow urine Conc.- 60% (G<sub>3</sub>) | 3.9                    | 3.9                    | 3.8                    | 3.9  |
| Cow urine Conc.- 80% (G<sub>4</sub>) | 3.7                    | 3.7                    | 3.6                    | 3.7  |
| Cow urine Conc.- 100% (G<sub>5</sub>) | 3.6                    | 3.5                    | 3.5                    | 3.5  |
| Mean             | 3.8                    | 3.7                    | 3.7                    |      |

| Duration (S) | Concentration (G) | G x S | SE±m | CD at 5 % |
|--------------|-------------------|-------|------|-----------|
|              |                   |       | 0.011| 0.042     |
|              |                   |       | 0.015| 0.042     |
|              |                   |       | 0.026| 0.078     |

Cow urine x soaking duration x growing media

SEm | 0.201

CD at 5 % | NS
Survival percentage of seedlings at 150 days after sowing

The results demonstrated that the effect of organic sources showed the significant effect on seedling survival percent at 150 days after sowing. The mean survival percentage of seedlings (77.0%) was recorded under $G_1S_1P_3$. Whereas, the minimum survival percentage of seedlings (52.50%) was recorded under control ($G_0S_3P_1$). The result has been reported by Ambika and Balakrishnan (2015) and Parmar et al., (2016) reported that the cow urine, PGPR are well known for better germination, seedling growth, vigour and suitable for commercial use for seed soaking before sowing to improve germination and seedling health.

On the basis of present investigation, it is concluded that the $G_1$ (20% cow urine), $S_1$ 24 hrs seed soaking duration and $P_3$ (6%) PGPR inoculation were superior over all other treatments in relation to all the viz; germination, growth and physiological parameters and the minimum results found in control in relation to all the growth parameters. Cow urine significantly encouraged germination, growth and survival of Custard apple seedlings. The interaction effect of cow urine, soaking duration and growing media showed non-significant effect on most of growth and physiological parameters of custard apple. It was concluded that $G_1S_1$ combination [seed soaked under cow urine concentration 20 % for 24 hrs] was the best combination with respect to growth and survival of custard seedling.

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