



# The Effect of Different Pre Sowing Treatments of Cow Urine, Soaking Duration, PGPR Applications and their Combinations on Seed Germination and Seedling Growth Parameters of Custard Apple (*Annona Squamosa* L.)

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

The study was conducted to evaluate "the effect of different pre sowing treatments of cow urine (20%,40%,60%,80%), soaking duration (24 hr, 48, 72 hr), PGPR (Plant growth promoting rhizobacteria) viz 0%,3%,6% applications and their combinations on seed germination and seedling growth parameters of Custard apple (*Annona Squamosa* L.)".The experiment was carried out during the year 2018-2019 at vegetable research farm Maharajpur, Department of Horticulture, College of Agriculture, JNKVV, Jabalpur (M.P.) with Asymmetrical Factorial Randomized block

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design with three replications and single seed treatment in net house condition. Germination parameters, vegetative growth parameters, physiological parameters, seedling vigour index were measured as per the standard procedures. The data obtained was subjected to statistical analysis in OPSTAT software. Cow urine, soaking duration and PGPR Media was found significant effect on days taken to start germination, days taken to 50% germination and germination percentage at 30 and 60 DAS. Maximum germination was recorded by the seeds treated with cow urine 20% for 24 hrs at 20 DAS, and 50 % germination at 35 DAS. The maximum shoot length, number of leaves, stem girth, LAD, LTR, Root length, Fresh and dry weight of shoot and root, seedling vigour index I and II, survival of seedlings were also observed in the seeds treated with cow urine 20% for 24 hrs. From this research, it was concluded that the treatment 20% cow urine for 24hrs soaking can be recommended for custard apple grown farmers to ensure sustainable and ecofriendly organic custard apple orchard with good yield.

**Keywords:** Custard apple; seed germination; PGPR; cow urine; organic production.

## 1. INTRODUCTION

“Custard apple (*Annona*), genus of about 160 species of small trees or shrubs of the family Annonaceae, native to the New World Tropics. *Annona squamosa* is native to the Tropical Americas and West Indies, but the exact origin is unknown. It is now the most widely cultivated of all the species of *Annona*, being grown for its fruit throughout the tropics and warmer subtropics, such as Indonesia, Thailand, Taiwan, and China as far north as Suzhou. It was introduced to southern Asia before 1590. It is naturalized as far north as southern Florida in the United States and as south as Bahia in Brazil, Bangladesh, and is an invasive species in some areas Custard apples are of local importance as traditional medicines, and several species are commercially grown for their edible fruits” [1]. “It has several synonyms such as Sitaphal, Sharifa, 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 flavor. 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 /100 g besides high nutritive value, it has also a high medicinal value” [2].

Members of the genus are typically evergreen or semi deciduous plants and cannot tolerate frost. The leaves can be leathery or hairy and are generally ovate with smooth margins. The unusual flowers feature six to eight fleshy curved petals in two whorls and numerous stamens and pistils. The fruits are often scaly and succulent and are sometimes segmented [3-6].

“Aggregate and soft fruits form from the numerous and loosely united pistils of a flower which become enlarged and mature into fruits which are distinct from fruits of other species of genus (and more like a giant raspberry instead). The round or heart-shaped greenish yellow, ripened aggregate fruit is pendulous on a thickened stalk; 5 centimetres (2.0 in) to 10 centimetres (3.9 in) in diameter with many round protuberances and covered with a powdery bloom. Fruits are formed of loosely cohering or almost free carpels (the ripened pistils)” [7].

The pulp is white tinged yellow, edible and sweetly aromatic. Each carpel containing an oblong, shiny and smooth, dark brown to black, 1.3 centimetres (0.51 in) to 1.6 centimetres (0.63 in) long seed. It is quite a prolific bearer, and it will produce fruit in as little as two to three years. A five-year-old tree can produce as many as 50 sugar apples. Poor fruit production has been reported in Florida because there are few natural pollinators (honeybees have a difficult time penetrating the tightly closed female flowers); however, hand pollination with a natural fibre brush is effective in increasing yield. In traditional Indian, Thai, and American medicine, the leaves are used in a decoction to treat dysentery and urinary tract infection. In traditional Indian medicine, they are also crushed and applied to wounds [8-11].

In Mexico, the leaves are rubbed on floors and put in hen's nests to repel lice. In Haiti the fruit is known as Cachiman and is used to make juice. In Lebanon and Syria, it is made into a variety of deserts and referred to as 'ashta'. “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 propagated 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 ripening 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” [12].

“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” [12].

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.

### 2.1 Treatment Details

Chart 1. The various factors with soaked time and PGPR

| Factor A                       | (Factor B Soaking Duration) | Factor C (PGPR)   |
|--------------------------------|-----------------------------|-------------------|
| G <sub>0</sub> Tap Water-100ml | S <sub>1</sub> 24 hours     | P <sub>1</sub> 0% |
| G <sub>1</sub> Cow Urine-20%   | S <sub>2</sub> 48 hours     | P <sub>2</sub> 3% |
| G <sub>2</sub> Cow Urine-40%   | S <sub>3</sub> 72 hours     | P <sub>3</sub> 6% |
| G <sub>3</sub> Cow Urine-60%   |                             |                   |
| G <sub>4</sub> Cow Urine-80%   |                             |                   |
| G <sub>5</sub> Cow Urine-100%  |                             |                   |

“This has diverted the attention once again towards, 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, Carboic Calcium, Salt, Vitamins, Lactose, Enzyme, Water, Creatin, Aurum Hydroxide etc. The enough work has been done on seed germination with use of PGR's and growing media but there is meagre work on seed treatment with organic matter like cow urine with soaking period and incorporation of plant growth promoting Rhizobacteria in growing media” [12]. In this context, the utilization of plant growth promoting Rhizobacteria (PGPR) may help to develop an environmentally biological approach for managing fungal diseases and enhancing the plant health resulting increase the yield.

### 2. MATERIALS AND METHODS

The experiment was conducted at vegetable Research Farm, Maha Rajpur, Department of Horticulture, JNKVV, Jabalpur (M.P.) during 2018-2019. Jabalpur is situated in “Kymore Platue 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 analyzed” [12].

### 3. METHOD OF PREPARATION AND APPLICATION

The treatments consistent of cow urine (20%,40%,60%,80%), soaking duration (24 hr, 48, 72 hr), PGPR (Plant growth promoting rhizobacteria) viz 0%,3%,6% applications and their combinations and control were replicated thrice in asymmetrical factorial randomized block design. Uniform size fully ripened fruits of custard apple were collected for seed extraction. Extracted seeds were washed in running water and dried under shade for 1 hour. Before drying of seeds, they were dipped in water to remove the dead floating seeds. Non - viable and dead seeds are generally light in weight, hence, they float on water. Other seeds, which settled at the bottom of the bucket, were considered viable were used for sowing. the collected seed were kept in 500 ml beaker for seed soaking for 24,48 and 72 hours. Freshly prepared solution of cow urine in different concentrations was added in each glass beaker as per treatment separately. treated seeds of custard apple were sown under 24,48 and 72 hours in polythene bags of 20x10 size filled with soil.10 seed per poly bags was sown at 2-3 cm depth on 25<sup>th</sup> august 2018. The observations regarding germination parameter viz. days taken to start germination, growth parameter viz. Plant height (cm), days taken to 50% germination, speed of germination, growth parameter viz. Plant height (cm), Number of leaves per seedling, number of roots, Length of roots, Fresh and dry weight of shoots and roots, Leaf area index and leaf area duration during 120-150 days, Survival % of seedlings at 150 days after sowing. For which five plants were randomly selected for observations and mean value was computed.

### 4. RESULTS AND DISCUSSION

#### 4.1 Effect on Germination

##### 4.1.1 DAS taken to start germination

Days taken to start germination were slightly better in the treatments having cow urine and their respective soaking durations, and the difference between them was significant. The minimum days of 20.1 were taken to germinate the seed of custard apple under G<sub>1</sub>S<sub>1</sub> (20% cow urine and 24 hrs soaking duration). Whereas the maximum days taken under G<sub>0</sub>S<sub>3</sub> 29.6 days to start germination.

##### 4.1.2 DAS taken to 50% germination

Regarding days taken to 50% germination of Annona seedlings as affected by different studied treatments showed such treatments significantly respond to the different investigated treatments. Herein, minimum days taken to 50% germination were recorded with 20% cow urine. Followed by 40%, 60%, 80%, and 100% cow urine treatments. In case of soaking duration 24 hrs duration found most significant effect on days taken to 50% germination. The least significant of days taken to 50% germination recorded with untreated trees (control) and in turn it was the inferior in this respect.

##### 4.1.3 Speed of germination

Referring to the relation between speed of germination and the different pre sowing treatments of Annona seedlings investigated treatments, indicated that the combine effect of cow urine and soaking duration, PGPR showed non-significant effect on speed of germination. Seeds treated with 20% cow urine soaked for 24 hrs showed highest speed in respect of germination. On the other way around, the reverse was true with those untreated seeds with any of the investigated treatments (control).

### 4.2 Growth Parameters

#### 4.2.1 No. of leaves

Data dealing with number of leaves of Annona seedlings as impacted by the different investigated treatments. It was quite clear that there were remarkable significant differences among the investigated treatments. Maximum number of leaves at 60, 90,120 and 150 days was recorded under under G<sub>1</sub>S<sub>1</sub> (20% cow urine and 24 hrs soaking duration). Whereas, Minimum values were recorded in control followed by G<sub>5</sub>S<sub>3</sub> (100% cow urine and 72 hrs soaking duration).

#### 4.2.2 Girth of stem

Concerning stem girth of custard apple seedling as affected by different studied treatments. Interactions of (cow urine x soaking duration), (cow urine x growing media) and (cow urine x soaking duration x growing media), showed significant effect on stem girth at all the stages. The maximum stem girth of 2.0, 2.4, 2.7, 3.1 mm was recorded under G<sub>1</sub>S<sub>1</sub>. Whereas the minimum stem girth was recorded seed soaked in tape

water for 72 hrs. Similar findings were also reported by Taiwo [13].

#### 4.2.3 Number of roots

With respect to the response of roots/seedling of custard- apple as impacted by different pre-sowing treatments cleared that there were significant differences between the investigated treatments in relation to number of roots/seedlings. Furthermore, the seeds which were treated with 20 % cow urine for 24 hrs reflected an acceptable increment in number of roots/seedling and in turn other treatments ranked first in this respect. In addition, treated seeds with

other treatments 40 % cow urine for 24 hrs came after the abovementioned treatment (G<sub>1</sub>S<sub>1</sub>) and an-subsequently occupied the second rank in this respect. On the other way around, the reverse was true with untreated seeds (Control) which exhibited the lowest number of roots/seedlings.

#### 4.2.4 Length of roots

Data concerning the relationship between length of roots of Custard apple seedlings and the different investigated treatments indicated that root length was significantly respond to the studied treatments. Furthermore, the seeds

**Table 1. Effect of different pre sowing treatments of cow urine (20%,40%,60%,80%), soaking duration (24 hr, 48, 72 hr), PGPR (Plant growth promoting rhizobacteria) viz 0%,3%,6% applications and their combinations on days taken to start seed germination of custard apple (*Annona squamosa* L.) Seedlings**

| 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> ) | 24.0                           | 23.6                        | 22.7                        | 23.4  |
| Soaking duration -48 hr. (S <sub>2</sub> ) | 25.0                           | 24.7                        | 24.3                        | 24.6  |
| Soaking duration -72 hr. (S <sub>3</sub> ) | 27.5                           | 26.9                        | 25.8                        | 26.7  |
| Mean                                       | 25.5                           | 25.1                        | 24.3                        |       |
|  | Growing media (P)              |                             | Soaking duration (S)        | S x P |
| SEm±                                       | 0.018                          | 0.108                       |                             | 0.187 |
| CD at 5 %                                  | 0.303                          | 0.303                       |                             | 0.540 |
| 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> )               | 29.0                           | 28.6                        | 28.3                        | 28.6  |
| Cow urine Conc.- 20% (G <sub>1</sub> )     | 23.2                           | 22.3                        | 21.4                        | 22.3  |
| Cow urine Conc.- 40% (G <sub>2</sub> )     | 24.8                           | 23.8                        | 22.3                        | 23.4  |
| Cow urine Conc.- 60% (G <sub>3</sub> )     | 24.9                           | 23.8                        | 23.7                        | 24.2  |
| Cow urine Conc.- 80% (G <sub>4</sub> )     | 25.5                           | 25.2                        | 24.7                        | 25.1  |
| Cow urine Conc.- 100% (G <sub>5</sub> )    | 26.3                           | 26.2                        | 25.2                        | 26.0  |
| Mean                                       | 25.5                           | 25.1                        | 24.3                        |       |
|  | Growing media (P)              |                             | Concentration (G)           | G x P |
| SEm±                                       | 0.153                          | 0.303                       |                             | 0.264 |
| CD at 5 %                                  | 0.428                          | 0.108                       |                             | 0.771 |
| 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> )               | 27.7                           | 28.7                        | 29.6                        | 28.6  |
| Cow urine Conc.- 20% (G <sub>1</sub> )     | 20.1                           | 22.1                        | 24.7                        | 22.3  |
| Cow urine Conc.- 40% (G <sub>2</sub> )     | 21.5                           | 23.2                        | 25.6                        | 23.4  |
| Cow urine Conc.- 60% (G <sub>3</sub> )     | 22.8                           | 23.7                        | 26.1                        | 24.2  |
| Cow urine Conc.- 80% (G <sub>4</sub> )     | 23.8                           | 24.6                        | 26.8                        | 25.1  |
| Cow urine Conc.- 100% (G <sub>5</sub> )    | 24.7                           | 25.6                        | 27.6                        | 26.0  |
| Mean                                       | 23.4                           | 24.6                        | 26.7                        |       |
|  | Duration (S)                   |                             | Concentration (G)           | G x S |
| SEm±                                       | 0.108                          | 0.153                       |                             | 0.264 |
| CD at 5 %                                  | 0.303                          | 0.428                       |                             | 0.741 |

**Table 2. Effect of different pre sowing treatments of cow urine (20%,40%,60%,80%), soaking duration (24 hr, 48, 72 hr), PGPR (Plant growth promoting rhizobacteria) viz 0%,3%,6% applications and their combinations on days taken to 50% 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> ) | 43.3                           | 42.8                        | 41.2                        | 42.4  |
| Soaking duration -48 hr. (S <sub>2</sub> ) | 45.3                           | 44.9                        | 44.0                        | 44.7  |
| Soaking duration -72 hr. (S <sub>3</sub> ) | 47.0                           | 45.8                        | 45.6                        | 46.1  |
| Mean                                       | 45.2                           | 44.5                        | 43.6                        |       |
|  | Growing media (P)              |                             | Soaking duration (S)        | S x P |
| SEm±                                       | 0.441                          | 0.441                       |                             | 0.763 |
| CD at 5 %                                  | 1.236                          | 1.236                       |                             | 1.118 |

|   | 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> )            | 47.6                           | 47.5                        | 47.1                        | 47.4  |
| Cow urine Conc.- 20% (G <sub>1</sub> )  | 42.6                           | 41.5                        | 36.7                        | 40.2  |
| Cow urine Conc.- 40% (G <sub>2</sub> )  | 43.5                           | 42.7                        | 42.6                        | 43.0  |
| Cow urine Conc.- 60% (G <sub>3</sub> )  | 44.4                           | 43.8                        | 43.8                        | 44.1  |
| Cow urine Conc.- 80% (G <sub>4</sub> )  | 45.8                           | 45.0                        | 44.8                        | 45.2  |
| Cow urine Conc.- 100% (G <sub>5</sub> ) | 47.2                           | 46.6                        | 46.4                        | 46.7  |
| Mean                                    | 45.2                           | 44.5                        | 43.6                        |       |
|   | Growing media (P)              |                             | Concentration (G)           | G x P |
| SEm±                                    | 0.441                          | 0.623                       |                             | 1.080 |
| CD at 5 %                               | 1.236                          | 1.748                       |                             | 2.851 |

|   | 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> )            | 46.3                     | 47.7                     | 48.3                     | 47.4  |
| Cow urine Conc.- 20% (G <sub>1</sub> )  | 35.4                     | 41.7                     | 43.6                     | 40.2  |
| Cow urine Conc.- 40% (G <sub>2</sub> )  | 41.4                     | 42.8                     | 44.6                     | 43.0  |
| Cow urine Conc.- 60% (G <sub>3</sub> )  | 42.4                     | 44.0                     | 45.7                     | 44.1  |
| Cow urine Conc.- 80% (G <sub>4</sub> )  | 43.6                     | 45.4                     | 46.6                     | 45.2  |
| Cow urine Conc.- 100% (G <sub>5</sub> ) | 45.5                     | 46.7                     | 48.0                     | 46.7  |
| Mean                                    | 42.4                     | 44.7                     | 46.1                     |       |
|   | Duration (S)             |                          | Concentration (G)        | G x S |
| SEm±                                    | 0.441                    | 0.623                    |                          | 1.080 |
| CD at 5 %                               | 1.748                    | 1.748                    |                          | 2.851 |

which were treated with 20 % cow urine for 24 hrs increased length of roots the highest length in cm comparing to other studied treatments followed by G<sub>2</sub>S<sub>1</sub> which come in second rank. Such trend was true during experimental season of study. On the other way around, the reverse was true with those untreated seeds with any of the investigated treatments (control).

#### 4.2.5 Fresh and dry weight of shoots and roots

With respect to the response of fresh and dry weight of both shoot and roots of Annona

seedlings as impacted by cow urine, soaking duration and seed inoculant PGPR cleared that there were significant differences between the investigated treatments in relation to fresh and dry weight of shoots and roots. Furthermore, the seeds which were treated with 20% cow urine for 24 hrs gave highest fresh and dry weight of Annona seedlings as compared with the other investigated treatments. Meanwhile, seed treated with 40%, 60% and 80% cow urine treatments not showed significant differences. Whereas, Minimum values were recorded in control followed by G<sub>5</sub>S<sub>3</sub> (100% cow urine + without PGPR).

#### 4.2.6 Leaf area index and leaf area duration during 120-150 days

The data pertaining to LAI and LAD during 120-150 days after sowing is presented in Table 4 and 5. The interaction of cow urine and soaking duration, cow urine and PGPR inoculation showed significant increment on LAI and LAD. The maximum LAI and LAD were noted under the treatment combination of G<sub>1</sub>S<sub>1</sub>. Whereas, minimum LAI and LAD were recorded under the treatment combination of G<sub>1</sub>P<sub>1</sub> and G<sub>1</sub>S<sub>3</sub>. The findings are supported by [14].

#### 4.2.7 Survival % of seedlings at 150 days after sowing

Regarding the impact of different investigated treatments on survivability of Annona seedlings, data in Table 6 indicated that the investigated parameter was highly significantly affected by different tested treatments. Herein, treated Annona seeds with cow urine (20%) with 24 hrs soaking duration proved to be the superior one in this respect as it maximized the investigated parameter (survival %) comparing to the other investigated treatments.

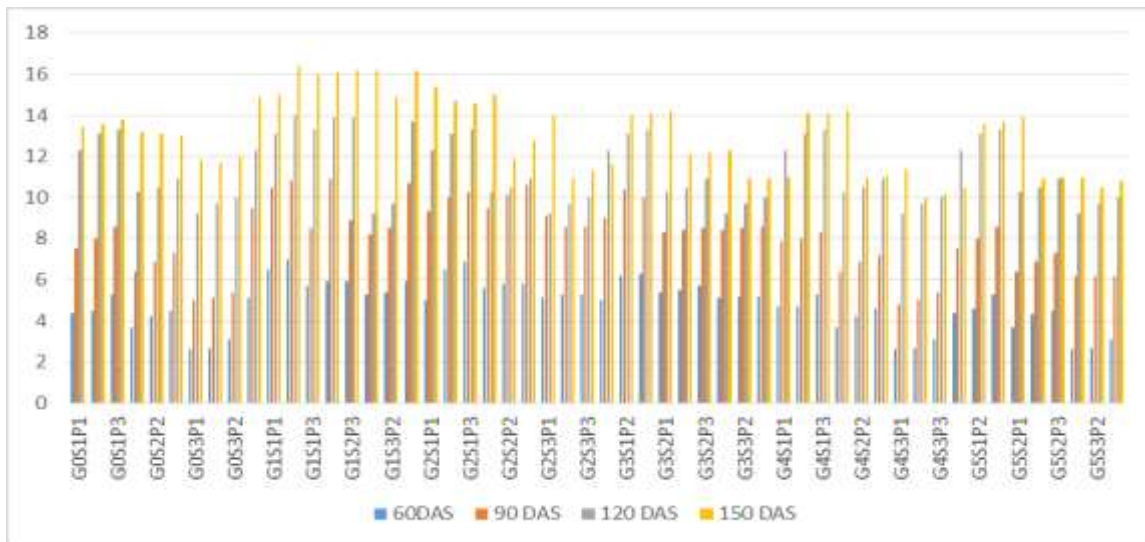


Fig. 1. Effect of different pre sowing treatments of cow urine (20%,40%,60%,80%), soaking duration (24 hr, 48, 72 hr), PGPR (Plant growth promoting rhizobacteria) viz 0%,3%,6% applications and their combinations on number of leaves

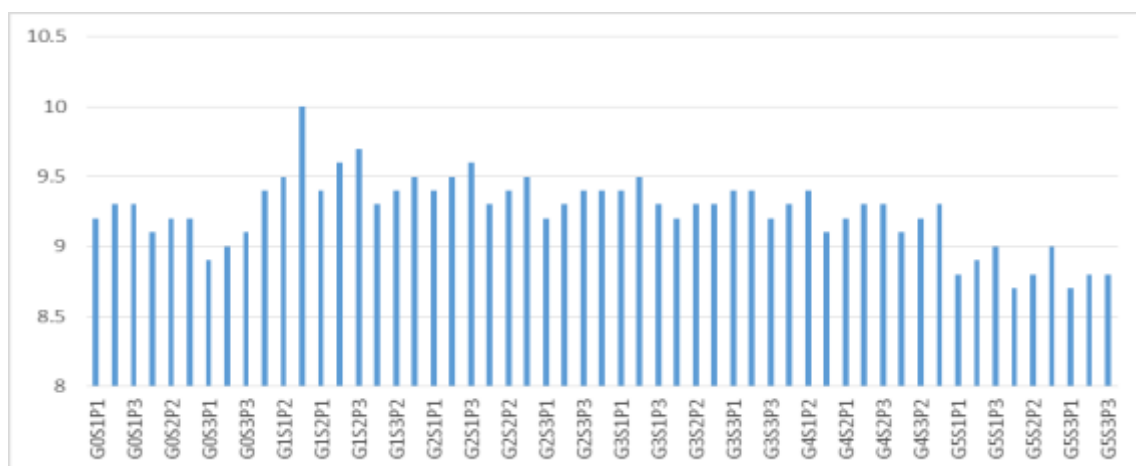


Fig. 2. Effect of different pre sowing treatments of cow urine (20%,40%,60%,80%), soaking duration (24 hr, 48, 72 hr), PGPR (Plant growth promoting rhizobacteria) viz 0%,3%,6% applications and their combinations on number of roots

**Table 3. Effect of different pre sowing treatments of cow urine (20%,40%,60%,80%), soaking duration (24 hr, 48, 72 hr), PGPR (Plant growth promoting rhizobacteria) viz 0%,3%,6% applications and their combinations on speed 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> ) | 0.51                           | 0.59                        | 0.78                        | 0.62  |
| Soaking duration -48 hr. (S <sub>2</sub> ) | 0.58                           | 0.59                        | 0.60                        | 0.59  |
| Soaking duration -72 hr. (S <sub>3</sub> ) | 0.50                           | 0.53                        | 0.54                        | 0.52  |
| Mean                                       | 0.55                           | 0.56                        | 0.62                        |       |
|  | Growing media (P)              |                             | Soaking duration (S)        | S x P |
| SEm±                                       | 0.051                          | 0.072                       |                             | 0.614 |
| CD at 5 %                                  | 0.165                          | NS                          |                             | NS    |
| Growing media                              |                                |                             |                             |       |
|  | 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> )               | 0.40                           | 0.43                        | 0.47                        | 0.43  |
| Cow urine Conc.- 20% (G <sub>1</sub> )     | 0.55                           | 0.54                        | 1.00                        | 0.71  |
| Cow urine Conc.- 40% (G <sub>2</sub> )     | 0.60                           | 0.62                        | 0.66                        | 0.63  |
| Cow urine Conc.- 60% (G <sub>3</sub> )     | 0.55                           | 0.57                        | 0.59                        | 0.58  |
| Cow urine Conc.- 80% (G <sub>4</sub> )     | 0.55                           | 0.57                        | 0.58                        | 0.57  |
| Cow urine Conc.- 100% (G <sub>5</sub> )    | 0.54                           | 0.55                        | 0.56                        | 0.55  |
| Mean                                       | 0.55                           | 0.56                        | 0.62                        |       |
|  | Growing media (P)              |                             | Concentration (G)           | G x P |
| SEm±                                       | 0.051                          | 0.072                       |                             | 0.126 |
| CD at 5 %                                  | 0.165                          | NS                          |                             | NS    |
| Soaking Duration                           |                                |                             |                             |       |
|  | 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> )               | 0.50                           | 0.43                        | 0.37                        | 0.43  |
| Cow urine Conc.- 20% (G <sub>1</sub> )     | 1.10                           | 0.61                        | 0.47                        | 0.71  |
| Cow urine Conc.- 40% (G <sub>2</sub> )     | 0.66                           | 0.62                        | 0.60                        | 0.63  |
| Cow urine Conc.- 60% (G <sub>3</sub> )     | 0.62                           | 0.56                        | 0.52                        | 0.58  |
| Cow urine Conc.- 80% (G <sub>4</sub> )     | 0.60                           | 0.59                        | 0.54                        | 0.57  |
| Cow urine Conc.- 100% (G <sub>5</sub> )    | 0.59                           | 0.55                        | 0.51                        | 0.55  |
| Mean                                       | 0.62                           | 0.59                        | 0.52                        |       |
|  | Duration (S)                   |                             | Concentration (G)           | G x S |
| SEm±                                       | 0.051                          | 0.072                       |                             | 0.126 |
| CD at 5 %                                  | NS                             | NS                          |                             | NS    |

**Table 4. Effect of different pre sowing treatments of cow urine (20%,40%,60%,80%), soaking duration (24 hr, 48, 72 hr), PGPR (Plant growth promoting rhizobacteria) viz 0%,3%,6% applications and their combinations on LAI (Leaf area index)**

| 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> ) | 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 |
| SEm±                                       | 0.011                          | 0.011                       |                             | 0.018 |
| CD at 5 %                                  | 0.033                          | 0.030                       |                             | 0.054 |



| <b>Growing media</b>                    |                                     |                                  |                                  |             |
|---|-------------------------------------|----------------------------------|----------------------------------|-------------|
|   | <b>Without PGPR (P<sub>1</sub>)</b> | <b>With PGPR (P<sub>2</sub>)</b> | <b>With PGPR (P<sub>3</sub>)</b> | <b>Mean</b> |
| 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                                 | 3.7                              | 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       |
| SEm±                                    | 0.011                               | 0.015                            |                                  | 0.026       |
| CD at 5 %                               | 0.033                               | 0.042                            |                                  | 0.078       |
| <b>Soaking Duration</b>                 |                                     |                                  |                                  |             |
|   | <b>24 hr. (S<sub>1</sub>)</b>       | <b>48 hr. (S<sub>2</sub>)</b>    | <b>72 hr. (S<sub>3</sub>)</b>    |             |
| 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       |
| SEm±                                    | 0.011                               | 0.015                            |                                  | 0.026       |
| CD at 5 %                               | 0.042                               | 0.042                            |                                  | 0.078       |

**Table 5. Effect of different pre sowing treatments of cow urine (20%,40%,60%,80%), soaking duration (24 hr, 48, 72 hr), PGPR (Plant growth promoting rhizobacteria) viz 0%,3%,6% applications and their combinations on LAD (Leaf area duration)**

| <b>Treatment</b>                           | <b>Growing media</b>                |                                  |                                  | <b>Mean</b> |
|--|-------------------------------------|----------------------------------|----------------------------------|-------------|
|  | <b>Without PGPR (P<sub>1</sub>)</b> | <b>With PGPR (P<sub>2</sub>)</b> | <b>With PGPR (P<sub>3</sub>)</b> |             |
| Soaking duration -24 hr. (S <sub>1</sub> ) | 1090.24                             | 1097.40                          | 1666.89                          | 1284.84     |
| Soaking duration -48 hr. (S <sub>2</sub> ) | 1120.27                             | 1122.97                          | 1127.40                          | 1123.55     |
| Soaking duration -72 hr. (S <sub>3</sub> ) | 1071.37                             | 1074.18                          | 1079.09                          | 1074.88     |
| Mean                                       | 1093.96                             | 1098.19                          | 1291.13                          |             |
|  | Growing media (P)                   | Soaking duration (S)             |                                  | S x P       |
| SEm±                                       | 106.97                              | 106.97                           |                                  | 185.28      |
| CD at 5 %                                  | 320.92                              | 320.92                           |                                  | 555.85      |
| <b>Growing media</b>                       |                                     |                                  |                                  |             |
|  | <b>Without PGPR (P<sub>1</sub>)</b> | <b>With PGPR (P<sub>2</sub>)</b> | <b>With PGPR (P<sub>3</sub>)</b> | <b>Mean</b> |
| Tape water (G <sub>0</sub> )               | 1054.62                             | 1054.90                          | 1055.60                          | 1055.04     |
| Cow urine Conc.- 20% (G <sub>1</sub> )     | 1130.93                             | 1139.77                          | 2255.84                          | 1508.84     |
| Cow urine Conc.- 40% (G <sub>2</sub> )     | 1102.71                             | 1111.77                          | 1124.84                          | 1113.12     |
| Cow urine Conc.- 60% (G <sub>3</sub> )     | 1098.78                             | 1103.45                          | 1118.96                          | 1107.02     |
| Cow urine Conc.- 80% (G <sub>4</sub> )     | 1088.73                             | 1094.39                          | 1102.70                          | 1095.27     |
| Cow urine Conc.- 100% (G <sub>5</sub> )    | 1085.20                             | 1087.72                          | 1088.86                          | 1087.26     |
| Mean                                       | 1093.96                             | 1098.79                          | 1291.13                          |             |
|  | Growing media (P)                   | Concentration (G)                |                                  | G x P       |
| SEm±                                       | 106.97                              | 151.28                           |                                  | 262.03      |
| CD at 5 %                                  | 320.92                              | 452.85                           |                                  | 786.09      |

|   | Soaking Duration         |                          |                          |         |
|---|--------------------------|--------------------------|--------------------------|---------|
|   | 24 hr. (S <sub>1</sub> ) | 48 hr. (S <sub>2</sub> ) | 72 hr. (S <sub>3</sub> ) |         |
| Tape water (G <sub>0</sub> )            | 1057.93                  | 1055.04                  | 1052.05                  | 1055.04 |
| Cow urine Conc.- 20% (G <sub>1</sub> )  | 2265.42                  | 1171.28                  | 1089.83                  | 1508.84 |
| Cow urine Conc.- 40% (G <sub>2</sub> )  | 1147.73                  | 1113.53                  | 1078.09                  | 1113.12 |
| Cow urine Conc.- 60% (G <sub>3</sub> )  | 1142.05                  | 1102.97                  | 1076.04                  | 1107.02 |
| Cow urine Conc.- 80% (G <sub>4</sub> )  | 1127.53                  | 1086.10                  | 1071.73                  | 1095.27 |
| Cow urine Conc.- 100% (G <sub>5</sub> ) | 1094.77                  | 1085.45                  | 1081.55                  | 1087.26 |
| Mean                                    | 1284.84                  | 1123.55                  | 1074.88                  |         |
|   | Duration (S)             | Concentration (G)        |                          | G x S   |
| SEm±                                    | 106.97                   | 151.28                   |                          | 262.03  |
| CD at 5 %                               | 320.92                   | 452.85                   |                          | 786.09  |

**Table 6. Effect of different pre sowing treatments of cow urine (20%,40%,60%,80%), soaking duration (24 hr, 48, 72 hr), PGPR (Plant growth promoting rhizobacteria) viz 0%,3%,6% applications and their combinations on Survival % of seedlings at 150 days after sowing**

| 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> ) | 64.6                           | 66.6                        | 68.5                        | 66.6  |
| Soaking duration -48 hr. (S <sub>2</sub> ) | 59.5                           | 61.0                        | 62.8                        | 61.1  |
| Soaking duration -72 hr. (S <sub>3</sub> ) | 59.3                           | 56.4                        | 57.6                        | 57.8  |
| Mean                                       | 61.1                           | 61.3                        | 63.0                        |       |
|  | Growing media (P)              | Soaking duration (S)        |                             | S x P |
| SEm±                                       | 0.042                          | 0.042                       |                             | 0.074 |
| CD at 5 %                                  | 0.119                          | 0.119                       |                             | 0.206 |

|   | 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> )            | 54.3                           | 55.3                        | 56.3                        | 55.3  |
| Cow urine Conc.- 20% (G <sub>1</sub> )  | 68.3                           | 70.3                        | 70.3                        | 70.9  |
| Cow urine Conc.- 40% (G <sub>2</sub> )  | 64.3                           | 66.8                        | 68.3                        | 66.5  |
| Cow urine Conc.- 60% (G <sub>3</sub> )  | 61.3                           | 62.6                        | 64.6                        | 62.8  |
| Cow urine Conc.- 80% (G <sub>4</sub> )  | 58.6                           | 59.6                        | 61.3                        | 59.8  |
| Cow urine Conc.- 100% (G <sub>5</sub> ) | 55.6                           | 57.0                        | 58.0                        | 56.8  |
| Mean                                    | 61.1                           | 61.3                        | 63.0                        |       |
|   | Growing media (P)              | Concentration (G)           |                             | G x P |
| SEm±                                    | 0.042                          | 0.060                       |                             | 0.104 |
| CD at 5 %                               | 0.119                          | 0.168                       |                             | 0.292 |

|   | Soaking Duration         |                          |                          |       |
|---|--------------------------|--------------------------|--------------------------|-------|
|   | 24 hr. (S <sub>1</sub> ) | 48 hr. (S <sub>2</sub> ) | 72 hr. (S <sub>3</sub> ) |       |
| Tape water (G <sub>0</sub> )            | 59.0                     | 55.6                     | 51.3                     | 55.3  |
| Cow urine Conc.- 20% (G <sub>1</sub> )  | 77.6                     | 67.6                     | 67.6                     | 70.9  |
| Cow urine Conc.- 40% (G <sub>2</sub> )  | 73.0                     | 66.3                     | 60.2                     | 66.5  |
| Cow urine Conc.- 60% (G <sub>3</sub> )  | 68.6                     | 63.0                     | 57.0                     | 62.8  |
| Cow urine Conc.- 80% (G <sub>4</sub> )  | 64.3                     | 60.0                     | 55.3                     | 59.8  |
| Cow urine Conc.- 100% (G <sub>5</sub> ) | 60.6                     | 55.3                     | 51.3                     | 56.8  |
| Mean                                    | 66.6                     | 61.6                     | 57.8                     |       |
|   | Duration (S)             | Concentration (G)        |                          | G x S |
| SEm±                                    | 0.042                    | 0.060                    |                          | 0.104 |
| CD at 5 %                               | 0.119                    | 0.168                    |                          | 0.292 |

## 5. CONCLUSION

Based on the findings of this study, it is concluded that the G1 (20% cow urine), S1 24 hr seed soaking duration, and P3 (6%) PGPR inoculation were superior to all other treatments in terms of germination, growth, and physiological parameters, with the control showing the lowest results in terms of all growth parameters. Cow urine promoted the germination, growth, and survival of Custard apple seedlings. The interaction effect of cow urine, soaking period, and growing media on most custard apple growth and physiological indicators was non-significant. It was determined that the G1S1 combination [seed soaked in 20% cow urine for 24 hours] was the optimum combination for custard seedling development and survival.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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