Original Research Article

"Influence of Different Pre Sowing Treatments on Seed Germination and Growth of Custard Apple (*Annona Squamosa* L.) Seedlings"

ABSTRACT

The study was conducted to evaluate "Influence of different pre sowing treatments of cow urine(20%,40%,60%,80%), soaking duration (24 hr, 48 hr , 72 hr) and PGPR (Plant growth promoting rhizobacteria) viz 0%,3%,6% applications on seed germination and growth of Custard apple (*Annona Squamosa* L.) Seedlings was carried out during the year 2018-2019 at vegetable research farm Maharajpur, Department of Horticulture, College of Agriculture, JNKVV, Jabalpur (M.P.) The experiment was laid out in Asymmetrical Factorial Randomized block 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 there standard procedures. The data obtained was subjected to the 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 % of germination at 30 and 60 DAS. Maximum germination recorded under G_1 (20%) treatment in 20.1 DAS when seed soaked for 24 hrs, 50 % germination at 35.4 DAS under G_1S_1, however with the application of cow urine when seed soaked for 24 hrs in 20 % cow urine shoot height, no. of leaves, girth of stem, LAD, LTR, Root length Fresh and dry weight of shoot and root, seedling vigour index I and II, survival of seedlings was found highest. From the previous research, it was concluded that custard apple seedlings resulted in better productivity and quality by the use of 20% cow urine and soaking duration 24 hours, and hence proved to be beneficial for sustainable development of farmers as they have provide better yield and also promote the organic production of fruit crops.

Key words: PGPR, Cow urine, organic production.

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. (Morton. 1987) 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 /100g besides high nutritive value, it has also a high medicinal value.

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.

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 pistels).

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.

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 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 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.

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, 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 meagre 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.

Chart 1 : The various factors with soaked time and PGPR.

FactorA	**Factor B(Soaked Time)**	**Factor C(PGPR)**
G_0Tap 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%		

METHODOLOGY

The experiment was conducted at vegetable Research Farm, Maharajpur, 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.

RESULTS & DISCUSSION

- **Germination parameters**

  - **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_1S_1 (20% cow urine and 24 hrs soaking duration). Whereas the maximum days taken under G_0S_3 29.6 days to start germination.

  - **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.

  - **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).

- **Growth parameters**
  - **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_1S_1 (20% cow urine and 24 hrs soaking duration). Whereas, Minimum values were recorded in control followed by G_5S_3 (100% cow urine and 72 hrs soaking duration).

  - **Girth of stem**

Concerning stem girth of custard apple seedling as affected by different studied treatments. Interactions of (cow urine × soaking duration), (cow urine × growing media) and (cow urine

× soaking duration × 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_1S_1. Whereas the minimum stem girth was recorded seed soaked in tape water for 72 hrs. Similar findings were also reported by Taiwo (2004).

- **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/ seedling. 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_1S_1) 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/ seedling.

- **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 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_2S_1 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).

- **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_5S_3 (100% cow urine + without PGPR).

- **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_1S_1. Whereas, minimum LAI and LAD were recorded under the treatment combination of G_1P_1 and G_1S_3. The findings are supported by (Peng et *al.*, 2013).

- **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

significant affected by different tested treatments. Herein, treated Annona seeds with cow urine (20%) with 24 hrs soaking duration proved to the superior one in this respect as it maximized the investigated parameter (survival %) comparing to the other investigated treatments.

Table 1 Effect of seed soaking duration, concentration of cow urine and growing media on DAS taken to start germination

Treatment	Growing media			Mean
	Without PGPR (P_1)	With PGPR (P_2)	With PGPR (P_3)	
Soaking duration -24 hr. (S_1)	24.0	23.6	22.7	23.4
Soaking duration -48 hr. (S_2)	25.0	24.7	24.3	24.6
Soaking duration -72 hr. (S_3)	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_1)	**With PGPR (P_2)**	**With PGPR (P_3)**	**Mean**
Tape water (G_0)	29.0	28.6	28.3	28.6
Cow urine Conc.- 20% (G_1)	23.2	22.3	21.4	22.3
Cow urine Conc.- 40% (G_2)	24.8	23.8	22.3	23.4
Cow urine Conc.- 60% (G_3)	24.9	23.8	23.7	24.2
Cow urine Conc.- 80% (G_4)	25.5	25.2	24.7	25.1
Cow urine Conc.- 100% (G_5)	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_1)	**48 hr. (S_2)**	**72 hr. (S_3)**	**Mean**
Tape water (G_0)	27.7	28.7	29.6	28.6
Cow urine Conc.- 20% (G_1)	20.1	22.1	24.7	22.3
Cow urine Conc.- 40% (G_2)	21.5	23.2	25.6	23.4
Cow urine Conc.- 60% (G_3)	22.8	23.7	26.1	24.2
Cow urine Conc.- 80% (G_4)	23.8	24.6	26.8	25.1
Cow urine Conc.- 100% (G_5)	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 seed soaking duration, concentration of cow urine and growing media on DAS taken to 50 % germination

Treatment	Growing media			Mean
	Without PGPR (P_1)	With PGPR (P_2)	With PGPR (P_3)	
Soaking duration -24 hr. (S_1)	43.3	42.8	41.2	42.4
Soaking duration -48 hr. (S_2)	45.3	44.9	44.0	44.7
Soaking duration -72 hr. (S_3)	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				
	Without PGPR (P_1)	**With PGPR (P_2)**	**With PGPR (P_3)**	**Mean**
Tape water (G_0)	47.6	47.5	47.1	47.4
Cow urine Conc.- 20% (G_1)	42.6	41.5	36.7	40.2
Cow urine Conc.- 40% (G_2)	43.5	42.7	42.6	43.0
Cow urine Conc.- 60% (G_3)	44.4	43.8	43.8	44.1
Cow urine Conc.- 80% (G_4)	45.8	45.0	44.8	45.2
Cow urine Conc.- 100% (G_5)	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				
	24 hr. (S_1)	**48 hr. (S_2)**	**72 hr. (S_3)**	**Mean**
Tape water (G_0)	46.3	47.7	48.3	47.4
Cow urine Conc.- 20% (G_1)	35.4	41.7	43.6	40.2
Cow urine Conc.- 40% (G_2)	41.4	42.8	44.6	43.0
Cow urine Conc.- 60% (G_3)	42.4	44.0	45.7	44.1
Cow urine Conc.- 80% (G_4)	43.6	45.4	46.6	45.2
Cow urine Conc.- 100% (G_5)	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

Table .3 Effect of seed soaking duration, concentration of cow urine and growing media on speed of germination

Treatment	Growing media			Mean
	Without PGPR (P_1)	With PGPR (P_2)	With PGPR (P_3)	
Soaking duration -24 hr. (S_1)	0.51	0.59	0.78	0.62
Soaking duration -48 hr. (S_2)	0.58	0.59	0.60	0.59
Soaking duration -72 hr. (S_3)	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				
	Without PGPR (P_1)	With PGPR (P_2)	With PGPR (P_3)	Mean
Tape water (G_0)	0.40	0.43	0.47	0.43
Cow urine Conc.- 20% (G_1)	0.55	0.54	1.00	0.71
Cow urine Conc.- 40% (G_2)	0.60	0.62	0.66	0.63
Cow urine Conc.- 60% (G_3)	0.55	0.57	0.59	0.58
Cow urine Conc.- 80% (G_4)	0.55	0.57	0.58	0.57
Cow urine Conc.- 100% (G_5)	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				
	24 hr. (S_1)	48 hr. (S_2)	72 hr. (S_3)	
Tape water (G_0)	0.50	0.43	0.37	0.43
Cow urine Conc.- 20% (G_1)	1.10	0.61	0.47	0.71
Cow urine Conc.- 40% (G_2)	0.66	0.62	0.60	0.63
Cow urine Conc.- 60% (G_3)	0.62	0.56	0.52	0.58
Cow urine Conc.- 80% (G_4)	0.60	0.59	0.54	0.57
Cow urine Conc.- 100% (G_5)	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

Effect of seed soaking duration, concentration of cow urine and growing media on number of leaves

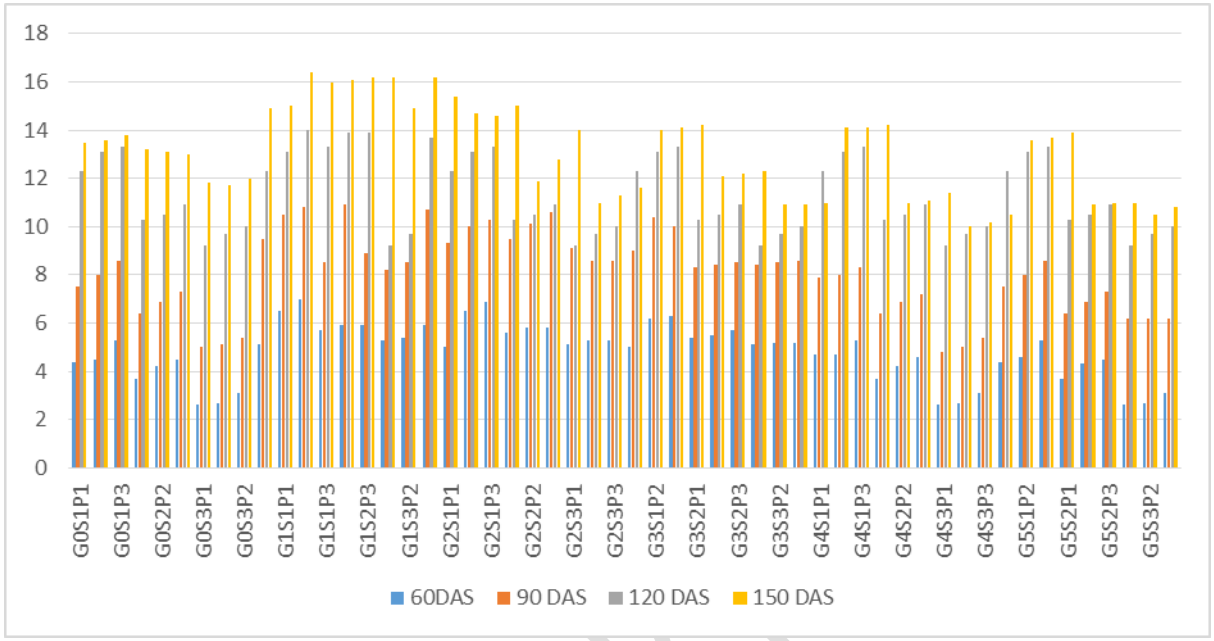

Fig. 1 Interaction effects of cow urine, soaked duration and PGPR on number of leaves

Effect of seed soaking duration, concentration of cow urine and growing media on number of roots

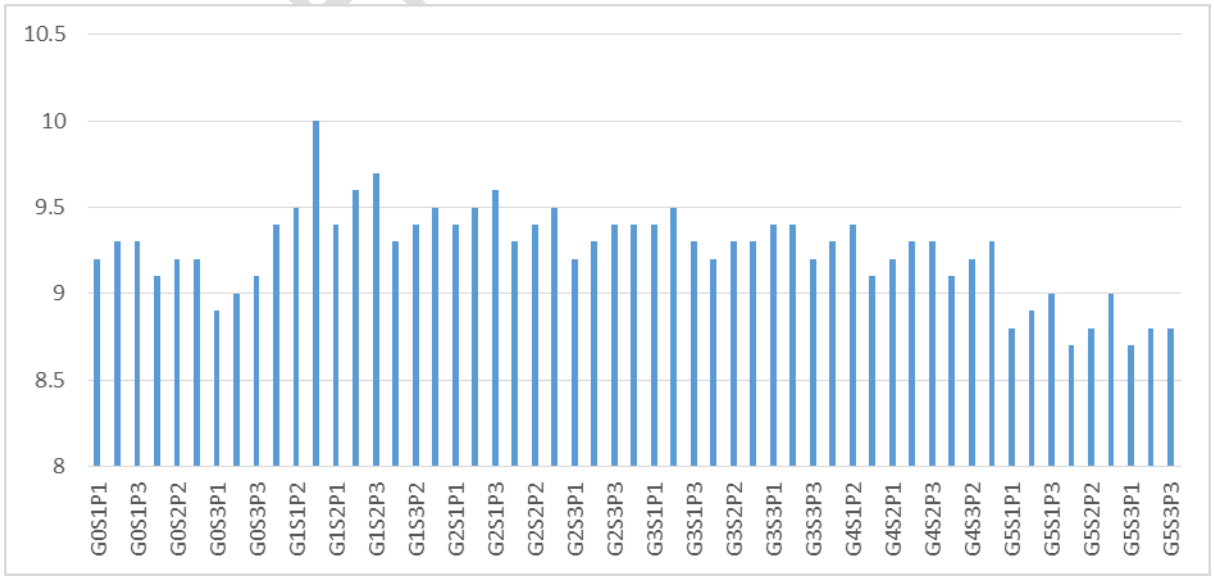

Fig. 2 Interaction effects of cow urine, soaked duration and PGPR on root length.

Table 4 Effect of seed soaking duration, concentration of cow urine and growing media on LAI (Leaf area index)

Soaking duration -24 hr. (S_1) Soaking duration -48 hr. (S_2)	Growing media			Mean
	Without PGPR (P_1)	**With PGPR (P_2)**	**With PGPR (P_3)**	
Soaking duration -72 hr. (S_3)	3.7	3.8	3.8	3.8
	3.7	3.7	3.7	3.7
	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
Growing media				
	Without PGPR (P_1)	**With PGPR (P_2)**	**With PGPR (P_3)**	**Mean**
Tape water (G_0)	3.4	3.5	3.5	3.5
Cow urine Conc.- 20% (G_1)	3.9	4.0	4.1	4.0
Cow urine Conc.- 40% (G_2)	3.9	3.9	3.9	3.9
Cow urine Conc.- 60% (G_3)	3.8	3.9	3.9	3.9
Cow urine Conc.- 80% (G_4)	3.7	3.7	3.8	3.7
Cow urine Conc.- 100% (G_5)	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
Soaking Duration				
	24 hr. (S_1)	**48 hr. (S_2)**	**72 hr. (S_3)**	
Tape water (G_0)	3.5	3.5	3.4	3.5
Cow urine Conc.- 20% (G_1)	4.0	3.9	3.9	4.0
Cow urine Conc.- 40% (G_2)	3.9	3.9	3.8	3.9
Cow urine Conc.- 60% (G_3)	3.9	3.9	3.8	3.9
Cow urine Conc.- 80% (G_4)	3.7	3.7	3.6	3.7
Cow urine Conc.- 100% (G_5)	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 seed soaking duration, concentration of cow urine and growing media on LAD (Leaf area duration)

Treatment	Growing media			Mean
	Without PGPR (P_1)	With PGPR (P_2)	With PGPR (P_3)	
Soaking duration -24 hr. (S_1)	1090.24	1097.40	1666.89	1284.84
Soaking duration -48 hr. (S_2)	1120.27	1122.97	1127.40	1123.55
Soaking duration -72 hr. (S_3)	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
Growing media				
	Without PGPR (P_1)	**With PGPR (P_2)**	**With PGPR (P_3)**	**Mean**
Tape water (G_0)	1054.62	1054.90	1055.60	1055.04
Cow urine Conc.- 20% (G_1)	1130.93	1139.77	2255.84	1508.84
Cow urine Conc.- 40% (G_2)	1102.71	1111.77	1124.84	1113.12
Cow urine Conc.- 60% (G_3)	1098.78	1103.45	1118.96	1107.02
Cow urine Conc.- 80% (G_4)	1088.73	1094.39	1102.70	1095.27
Cow urine Conc.- 100% (G_5)	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_1)	**48 hr. (S_2)**	**72 hr. (S_3)**	
Tape water (G_0)	1057.93	1055.04	1052.05	1055.04
Cow urine Conc.- 20% (G_1)	2265.42	1171.28	1089.83	1508.84
Cow urine Conc.- 40% (G_2)	1147.73	1113.53	1078.09	1113.12
Cow urine Conc.- 60% (G_3)	1142.05	1102.97	1076.04	1107.02
Cow urine Conc.- 80% (G_4)	1127.53	1086.10	1071.73	1095.27
Cow urine Conc.- 100% (G_5)	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 seed soaking duration, concentration of cow urine and growing media on Survival % of seedlings at 150 days after sowing

Treatment	Growing media			Mean
	Without PGPR (P_1)	With PGPR (P_2)	With PGPR (P_3)	
Soaking duration -24 hr. (S_1)	64.6	66.6	68.5	66.6
Soaking duration -48 hr. (S_2)	59.5	61.0	62.8	61.1
Soaking duration -72 hr. (S_3)	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				
	Without PGPR (P_1)	**With PGPR (P_2)**	**With PGPR (P_3)**	**Mean**
Tape water (G_0)	54.3	55.3	56.3	55.3
Cow urine Conc.- 20% (G_1)	68.3	70.3	70.3	70.9
Cow urine Conc.- 40% (G_2)	64.3	66.8	68.3	66.5
Cow urine Conc.- 60% (G_3)	61.3	62.6	64.6	62.8
Cow urine Conc.- 80% (G_4)	58.6	59.6	61.3	59.8
Cow urine Conc.- 100% (G_5)	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_1)	**48 hr. (S_2)**	**72 hr. (S_3)**	
Tape water (G_0)	59.0	55.6	51.3	55.3
Cow urine Conc.- 20% (G_1)	77.6	67.6	67.6	70.9
Cow urine Conc.- 40% (G_2)	73.0	66.3	60.2	66.5
Cow urine Conc.- 60% (G_3)	68.6	63.0	57.0	62.8
Cow urine Conc.- 80% (G_4)	64.3	60.0	55.3	59.8
Cow urine Conc.- 100% (G_5)	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

CONCLUSION

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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