Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(6): 552-556
Received: 16-09-2020
Accepted: 21-10-2020

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Effect of organic and inorganic sources on seed germination, growth and survival of Custard apple (*Annona squamosa* L.) seedlings

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Abstract
A field experiment was conducted at Fruit Research Station, Imalia, College of Agriculture, JNKVV Jabalpur during 2016-17 to assess the effect of organic and inorganic sources on seed germination, growth and survival of Custard apple (*Annona squamosa* L.) seedlings. The treatments consistent of GA_3, cow dung and cow urine with their different concentration were arranged in randomized block design with three replications. The significant effect and higher value of all parameters were observed with GA_3 over rest of the treatments. Moreover, looking to movement towards organic farming the pre sowing seed soaking with organic sources also gave significant effect and value of different parameters *viz*. day taken to start germination (38 days), to attain 50% germination 52.25 days), Plant height (11.63cm), number of leaves per seedling (11.87), stem girth (3.68mm), seedling length (22.20cm), root length (10.67cm), number of roots per seedling (42.00), Chlorophyll Content Index (14.77), LAI (0.86), LAD (1696.09), LTR (58.43),EI (0.53), fresh and dry weight of shoots (3.25 and 0.61g), fresh and weight of roots (0.73 and 0.40g), seedling vigour index-I (1731.25), seedling vigour index-II (79.35), seedling survival (77.50%) were recorded with seed socked in cow dung (100%) at 150 days after sowing which was closely followed by cow urine (100%).

Keywords: Organic sources, cow dung, cow urine, seed germination

Introduction
Custard apple (*Annona squamosa* L.) also known as Sitaphal belongs to family Annonaceae. It is indigenous to tropical America and is now grown in all over the world. In India, the Custard apples are very popular in Deccan Plateau and are grown commercially on smaller scale in Madhya Pradesh, Andhra Pradesh, Bihar, Gujrat, Rajasthan, Tamilnadu, Aasam, Karnataka, Orrisa and Maharastra (Anonymous, 2015) [1]. The plants are hardy, medium in growth and fruits are very delicious. Plants are generally classified as semi wild fruit by nature virtue of its spontaneous spread in forest, wastelands and other cultivated places. Fruits are considered for their medicinal value besides their industrial use in ice-cream, confectionary, certain milk products, making preserves as jam, jelly and other products. In general, Custard apple propagated by seed, but due to hard and thick seed coat, it requires about 35-50 days for germination (Torres and Sanchez, 1992) [20]. To get higher and proper germination with vigorous seedling growth, seed needs pre-treatments before sowing which helps in promotion of early and higher percentage of germination with healthy and vigorous seedlings (Chadha,2010) [4]. The use of plant growth regulators in proper concentration with scarification may regulate growth behavior in many fruit crops and pre-sowing treatment of growth regulators could lead to increase seed germination and enhancement of seedling growth. Seeds, without use of growth regulators showed poor response for germination and its growth. Plant growth regulators like GA_3, IBA and IAA enhance the germination and survival of seedlings. GA_3 issued for weakening of the seed coat so that the radical of the seedling can be broken through the seed coat. The prices of growth regulators have gone high and availability is also constants to overcome this crisis some traditional methods or alternatives for growth regulators are easily available to access for this purpose. This has diverted the attention once again towards the organics, now a day's cow urine and cow dung is used as a growth regulators. 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 Iron, urea, Uric acid, estrogen and progesterone which affect the inhibitory response to seed germination, shoot growth and seedling vigour (Dilrukshi, 2009) [7].

Material and Methods
A field experiment was conducted at Fruit Research Station, Imalia, College of Agriculture, JNKVV Jabalpur during 2016-17.

The treatments consistent of GA_3, cow dung and cow urine with their different concentration *viz*. T_1 - GA_3 (200 ppm), T_2 - Cow dung (100%), T_3 - Cow urine (100%), T_4 - Cow dung (25%)+Cow urine (75%),T_5 - Cow dung (50%)+Cow urine (50%), T_6 - Cow dung (75%)+Cow urine (25%), T_7 - Cow dung (25%)+Water(75%), T_8 - Cow dung (50%)+Water (50%),T_9 - Cow dung (75%)+Water (25%),T_{10} - Cow urine (25%)+ Water (75%),T_{11}- Cow urine (50%)+ Water (50%), T_{12} - Cow urine (75%)+Water (25%) and T_{13}-Control were replicated thrice in 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.

Required quantity 200 mg of GA_3 was weighed with the help of electronic balance. After weighing, GA_3 was transferred into glass beaker with the help of soft brush and added 1000 ml. distilled water to make solution of 200 ppm. Fresh cow urine and cow dung were measured in glass beaker separately and distil water was added to make the solution 25, 50, 75 and 100 percent as per treatments. The extracted seeds were kept in 1000 ml of glass beaker for seed soaking for 24 hrs. Freshly prepared solution of GA_3, cow dung and cow urine in different concentration was added in each glass beaker as per treatment separately. Black poly bags having length of 20 cm and diameter of 10 cm with 200 gauge thickness, were used and filled with the media comprising of soil+FYM+sand in the ratio of 3:1:1, respectively. One seed per poly bag was sown at 4-5 cm depth on 28th Nov. 2016.Watering and other operations were done as per requirement. The observations regarding germination parameter *viz*. days taken to start germination, days taken to 50% germination, growth parameter *viz*. Plant height (cm), Number of leaves per seedling, Girth of stem (mm), Length of seedling (cm), Root length (cm),Number of roots per seedling, Dry weight of shoots (g), Fresh weight of roots (g), Dry weight of roots (g), Seedling vigour index-I (cm),Seedling vigour index-II (g),Survival percent of seedling and physiological parameters *viz*. Chlorophyll Content Index, Leaf Area Index (LAI),Leaf Area Duration (LAD),Light Transmission Ratio (LTR),Energy Interception (EI) were recorded at 150 days after sowing. For which five plants were randomly selected for observations and mean value was computed.

Results and Discussion

The significant effect of GA_3 was observed in all the parameters under study over all the other treatments. However, organic sources also showed significant effect over control. Therefore, findings are briefly discussed and interpreted in support of the previous research findings pertaining to this investigation.

Effect on Germination

Seed soaked before sowing with 100% cow dung for 24 hours were required 38.00 days to start germination and 52.25 days attained to 50% germination of seed which is followed by 100% cow urine. The increase in germination percentage might be due to involvement of cow dung and cow urine in the activation of cytological enzymes which increase cell wall plasticity and better water absorption. These findings are supported by Deshpande *et al.* (2008) [6] and Parmar *et al.* (2016) [16].

The result indicated that when seed soaking with 200 ppm GA_3 for 24 hrs has taken the minimum days (36.00) to start germination and 52 days to attained 50% germination. The increase in germination was due to involvement of GA_3 in the activation of cytological enzymes along with increase cell wall plasticity and better water absorption. These findings are supported by Dhankhar and Singh (1996) [8].

Effect growth parameter

The plant height (11.63 cm), number of leaves (11.87), Stem girth (3.68 mm), length of seedling (22.20 cm), root length (10.67 cm) and number of root / seedling (42.00) were recorded at successive growth stages under the treatment 100% cow dung followed by 100% cow urine. Plant height might be influenced by cow dung and cow urine which induced the cell elongation process and quicker multiplication of cell after germination, ultimately increased the height of the plant. These results are supported by Rao (1975) [17] and Parmar *et al.* (2016) [16] and Palepad *et al.* (2016).

The maximum plant height (12.91 cm), number of leaves (13.75), Stem girth (4.01 mm), length of seedling (24.27 cm), root length (11.56 cm) and number of root / seedling (44.87). It was due to additional GA_3, activated α-amylase which digested the available carbohydrate into sugar so that energy and nutrition were easily available to faster the growing of seedlings. Increase in plant height due to pre sowing seed soaking in GA_3 has been reported by Shant and Rao (1973) [18]. GA_3 promote the growth of the plant by the promotion of cell elongation. These results showed that the application of plant growth regulators might be helpful for enhancing growth of seedling and reducing the time. The results are supported by Mobli and Baninasab (2008) [14] and Parmar *et al*. (2016) [16].

Chlorophyll Content Index

The present study reveals that the higher chlorophyll content index is normally associated with the higher quantum yield as long as the chlorophyll possessed the higher amount of active chlorophyll which absorbs the solar radiation more efficiently and converts into chemicals energy. In the present investigation GA_3 at 200 ppm recorded the maximum chlorophyll content index (19.49) and the minimum chlorophyll content index (10.92) was noted in control. In the present investigation of organic sources 100% cow dung recorded the maximum chlorophyll content index (19.49) whereas, the minimum chlorophyll content index (10.92) was noted in control. These findings are supported by Ilango *et al*. (1999) [11].

Leaf Area Index (LAI) and Leaf Area Duration (LAD)

Leaf Area is an important input in physiological and agronomic studies such as in various transpiration models, characterization of crop growth, LAI etc. LA and LAI decreased very markedly and rapidly at senescence and dropping of the lower leaves. The effect of organic and inorganic sources showed significant effect on Leaf Area Index and Leaf Area Duration. The maximum Leaf Area Index (1.13) and Leaf Area Duration (2215.62cm^2.days) were noted under treatment GA_3 at 200 ppm. Whereas, the minimum Leaf Area Index (0.53) and Leaf Area Duration (1056.25 cm^2.days) were obtained under treatment control. This was higher ascribed to higher magnitude increases in parameter associated with the LA. The finding was supported Munde and Gajbhiye (2010) [15].

Light Transmission Ratio (LTR) %

In the present study, significantly the minimum Light Transmission Ratio 52.96% at 150 days after sowing was computed in GA_3 at 200 ppm followed by 100% cow dung. The maximum Light Transmission Ratio 69.41% was recorded under control. The finding was supported by Munde and Gajbhiye (2010) [15]. The results were significantly correlated with LAI which exhibited the lowest and the highest transmission through the crop canopy is reflected in higher value of LAI, more canopy size more light interception and less transmission through the canopies Thakur and Kaur (2001) [19].

Energy interception

The present study revealed that the effect of organic and inorganic sources showed significant effect on Energy Interception. Significantly higher Energy Interception 0.53 at 150 days after sowing was computed in GA_3at 200ppm followed by 100% cow dung as compare to control. 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 develops leaf area rapidly. Similar findings were reported by Maddonni and Otegui (1996) [13].

Fresh and dry weight of shoots and roots (g)

The result indicated that the maximum fresh and dry weight of shoots (3.25 g and 0.61 g) and roots (0.73g and 0.40 g) respectively were obtained at 150 days after sowing under treatment 100% cow dung followed by 100% cow urine when seed sown before sowing for 24 hrs. The results are supported with the findings of Jadhav *et al.* (2015) [12], Parmar *et al.* (2016) [16] and Palepad *et al.* (2016). As regards inorganic sources, the maximum fresh and dry weight (3.30 g and 0.76 g) of shoots and roots (0.93g and 0.45 g) respectively were obtained at 150 days after sowing under pre-sowing seed soaking in GA_3 solution of 200 ppm. The results are supported with these findings of Choudhary and Chakrawar (1982) [5].

Seedling Vigour Index

The maximum value of seedling vigour index-I (1731.25cm) and seedling vigour index-II (79.35g) was recorded with soaking of seed in 100% cow dung for the period of 24 hrs which was closely followed by 100% cow urine which is superior to all other combination of cow dung and cow urine. The findings are supported by Gurung *et al.* (2014) [10] and Ambika and Balakrishanan (2015) [15].

As regard the inorganic source, GA_3 showed significant effect on seedling vigour index- I and seedling vigour index- II and the maximum seedling vigour index I (2086.50 cm) and seedling vigour index- II (96.25g) was recorded under GA_3 at 200 ppm and the minimum value was recorded under control. The findings are supported by Dhoran and Gudadhe (2012) [9].

Survival percent of seedlings

The effect of organic and inorganic sources showed significant effect on seedling survival percent at 150 days after sowing. The mean survival percentage of seedlings (77.50%) was recorded under 100% cow dung followed by 100% cow urine (75.00%). The result has been reported by Ambika and Balakrishanan (2015) [15] and Parmar *et al.* (2016) [16] reported that the cow dung and cow urine 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.

The results demonstrated that the effect of organic sources showed the significant effect on seedling survival percent at 150 days after sowing. The maximum mean survival percentage of seedlings (85.00%) was recorded under seed soaking with 200 ppm GA_3 whereas; the minimum survival percentage of seedlings (52.50%) was recorded under control. GA_3 is well known for better germination, seedling growth and vigour are highly suitable for commercial cultivation and their germination and seedling health can be improved.

Table 1: Effect of organic and inorganic sources on germination and growth of Custard apple seedlings at 150 DAS

Symbols	Treatment details	Germination (DAS)	Days to 50% germination	Plant Height (cm)	Number of leaves	Girth of stem (mm)	Seedling Length (cm)	Root length (cm)	No Roots / seedling
T_1	GA_3 (200 ppm)	36.00	52.00	12.91	13.75	4.01	24.47	11.56	44.87
T_2	Cow dung (100%)	38.00	52.25	11.63	11.87	3.68	22.20	10.67	42.00
T_3	Cow urine (100%)	40.00	53.75	11.52	11.00	3.48	22.08	10.67	37.87
T_4	Cow dung (25%) + Cow urine(75%)	40.00	55.00	11.46	10.75	3.39	21.98	10.62	36.25
T_5	Cow dung (50%) + Cow urine(50%)	40.00	55.50	11.37	10.50	3.39	21.73	10.51	35.62
T_6	Cow dung (75%) + Cow urine(25%)	40.75	60.00	11.20	10.37	3.38	21.72	10.50	35.25
T_7	Cow dung (25%) + Water (75%)	41.75	63.00	10.65	10.12	3.27	20.66	10.01	34.12
T_8	Cow dung (50%) + Water (50%)	41.50	61.00	11.06	10.25	3.27	21.62	10.42	34.12
T_9	Cow dung (75%) + Water (25%)	41.25	60.50	11.10	10.25	3.30	21.70	10.46	34.25
T_{10}	Cow urine (25%) + Water (75%)	43.25	65.75	10.25	10.00	3.20	19.68	9.42	31.87
T_{11}	Cow urine (50%) + Water (50%)	42.75	64.50	10.28	10.00	3.25	20.07	9.93	32.12
T_{12}	Cow urine (75%) + Water (25%)	42.50	64.25	10.57	10.00	3.27	20.51	9.97	33.00
T_{13}	Control	50.25	67.00	10.07	9.37	3.02	19.58	9.07	31.75
SEm±		1.36	1.47	0.31	0.38	0.09	0.59	0.33	0.91
CD at 5%		3.93	4.25	0.89	1.10	0.24	1.69	0.96	2.63

Table 2: Effect of organic and inorganic sources on physiological parameters of Custard apple seedlings at 150 DAS

Symbols	Treatment details	Chlorophyll Content Index	Leaf Area Index	Leaf Area Duration during	Light Transmission Ratio	Energy Interception
T_1	GA_3 (200 ppm)	19.49	1.13	2215.62	52.96	0.53
T_2	Cow dung (100%)	14.77	0.86	1696.09	58.43	0.53
T_3	Cow urine (100%)	14.41	0.73	1461.71	61.52	0.48
T_4	Cow dung (25%) +Cow urine (75%)	14.06	0.67	1336.32	62.27	0.47
T_5	Cow dung (50%) + Cow urine (50%)	13.88	0.65	1268.74	62.77	0.46
T_6	Cow dung (75%) + Cow urine (25%)	12.45	0.61	1214.06	63.33	0.44

T_7	Cow dung (25%) + Water (75%)	11.91	0.57	1160.54	65.41	0.39
T_8	Cow dung (50%) + Water (50%)	11.92	0.58	1071.09	64.35	0.40
T_9	Cow dung (75%) + Water (25%)	12.43	0.64	1196.48	64.05	0.41
T_{10}	Cow urine (25%) + Water (75%)	11.21	0.54	1125.00	68.50	0.36
T_{11}	Cow urine (50%) + Water (50%)	11.45	0.57	1132.81	66.49	0.37
T_{12}	Cow urine (75%) + Water (25%)	11.61	0.57	1140.23	66.29	0.37
T_{13}	Control	10.92	0.53	1056.25	69.41	0.35
SEm±		1.03	0.04	70.79	2.99	0.04
CD at 5%		2.99	0.11	203.87	8.60	0.12

Table 3: Effect of organic and inorganic sources on vigour and seedlings survival of Custard apple

Symbols	Treatment details	Fresh weight of shoots (g)	Dry weight of shoots (g)	Fresh weight of roots (g)	Dry weight of roots(g)	Seedling vigour index -I(cm)	Seedling vigour index -II(g)	Seedling survival (%)
T_1	GA_3 (200 ppm)	3.30	0.76	0.93	0.45	2,086.50	96.25	85.00
T_2	Cow dung (100%)	3.25	0.61	0.73	0.40	1,731.25	79.35	77.50
T_3	Cow urine (100%)	3.19	0.59	0.71	0.36	1,662.62	70.67	75.00
T_4	Cow dung (25%) +Cow urine (75%)	3.11	0.57	0.70	0.36	1,601.37	67.35	72.50
T_5	Cow dung (50%) + Cow urine (50%)	3.06	0.56	0.67	0.35	1,392.12	57.02	65.00
T_6	Cow dung (75%) + Cow urine (25%)	3.05	0.55	0.66	0.30	1,410.12	55.25	65.00
T_7	Cow dung (25%) + Water (75%)	2.96	0.54	0.64	0.28	1,187.12	47.42	57.50
T_8	Cow dung (50%) + Water (50%)	2.99	0.54	0.64	0.29	1,304.25	50.55	60.00
T_9	Cow dung (75%) + Water (25%)	3.00	0.55	0.64	0.29	1,363.12	52.25	62.50
T_{10}	Cow urine (25%) + Water (75%)	2.68	0.52	0.51	0.26	1,050.25	43.80	55.00
T_{11}	Cow urine (50%) + Water (50%)	2.84	0.52	0.63	0.26	1,113.12	45.32	55.00
T_{12}	Cow urine (75%) + Water (25%)	2.94	0.53	0.63	0.28	1,163.87	47.55	57.50
T_{13}	Control	1.89	0.45	0.43	0.20	1,036.25	34.17	52.50
SEm±		0.14	0.03	0.02	0.02	63.34	3.54	2.27
CD at 5%		0.42	0.09	0.06	0.07	182.41	10.20	6.53

Acknowledgment
The first author is thankful to Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur authority for providing facilities and technical guidance during the course of investigation of research work.

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