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Roshni Singh Dahayat
Department of Horticulture, College of Agriculture, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh, India

Vijay Kumar Singh
Department of Horticulture, College of Agriculture, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh, India

Latesh Kumar
Department of Horticulture, College of Agriculture, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh, India

Sangha Payasi
Department of Soil Science, Mahatma Gandhi Chitrakoot Gramoday University Chitrakoot Satna, Madhya Pradesh, India

Corresponding Author:
Roshni Singh Dahayat
Department of Horticulture, College of Agriculture, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh, India

Effect of chemicals and bio agents on seed germination, vigour & seedling growth of custard apple (*Annona squamosa* L.) under net house condition

Roshni Singh Dahayat, Vijay Kumar Singh, Latesh Kumar and Sangya Payasi

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Abstract
The experiment entitled," Effect of chemicals and bio agents on seed germination, vigour and seedling growth of Custard Apple (*Annona squamosa* L.) under Net house condition" was carried out at Department of Horticulture, College of Agriculture, Rewa, (M.P.). Custard apple is a widely cultivated fruit species in India, valued for its taste and nutritional content. Originating from the West Indies, it has been extensively cultivated in Central America and Southern Mexico and it belongs to the Annonaceae family. Custard apple is typically propagated by seed. The hard seed coat of custard apple seeds requires 35-50 days for natural germination. To speed up the process, pre-sowing treatments like GA_3, cow urine and dung slurry, potassium nitrate, NAA, thiourea, and HCl or H_2SO_4 are used to break dormancy and promote germination. The experiment was laid out in Completely Randomized Design with three replications. The experiment comprised of fifteen treatments consisting of KNO_3 @ 1% and 2%, Thiourea @ 1% and 2%, Cow urine @ 10% and 20%, Cow dung slurry @ 10% and 20%, GA_3 @ 100 and 200 ppm, NAA @ 100 and 200 ppm for 24 hours and HCl and H_2SO_4 @ 0.1 % for 2 minutes. Result indicated that treatment GA_3 @ 200 ppm for 24 hours resulted in early and maximum seed germination (78.26%), increased height, stem diameter, no. of leaves, leaf area, fresh and dry weight of leaves and root, and a seedling vigour. Minimum value was found in control.

Keywords: Annona, cow dung, cow dung slurry, germination, Gibberellic Acid, HCl, KNO_3

Introduction
Custard apple (*Annona squamosa* L.) is also known as Sitaphal, Sugar apple, sharifa, sweet soap etc. which is grown in tropical and subtropical climates. It belongs to the Annonaceae family and is a native of the West Indies, but it has long been cultivated in Central America and Southern Mexico. There are more than 100 species in the genus, five of which have edible fruits species which includes *Annona squamosa* L. (Custard Apple), *Annona muricata* L. (Soursop), *Annona reticulata* L. (Bullock Heart, Ramphal), *Annona atemoya* L. (Lakshman phal) and *Annona cherimola* L. (Hanu-manphal). Among all the species Custard apple is the most popular monoecious fruit in India. According to Department of Agriculture and farmers welfare 2023-24 presently, in India custard apple grown in area of 52,000 ha. with production of 460,000 MT. Madhya Pradesh is 2nd largest producer of Custard Apple with area of 9.602 thousand ha. production of 116.27 thousand MT of fruit production (Anonymous, 2022-23) [2, 3]. Seed germination marks the initial phase of plant development, signifying the reactivation of embryo growth leading to the emergence of a young plant. The dormancy in seeds can be attributed to factors such as a tough and impermeable seed coat, germination inhibitors and irregular embryo development. The hard seed coat of custard apple seeds requires 35-50 days for natural germination. To enhance and ensure successful germination, pre-sowing treatments are essential. These treatments facilitate early germination and the growth of robust seedlings. To speed up the germination process, pre-sowing treatments like GA_3, cow urine and dung slurry, potassium nitrate, NAA, thiourea, HCl and H_2SO_4 are used to break dormancy and promote germination. Cow urine is emerging as a viable option that could potentially revolutionize the current scenario.

GA_3 breaks seed dormancy by activating gluconeogenic enzymes. Potassium nitrate enhances germination by improving nutrient absorption and water uptake. NAA promotes germination and seedling growth at low concentrations. Thiourea boosts seed germination, while HCl helps break the seed coat to reduce dormancy.

Materials and Methods

The experiment was carried out in Department of Horticulture, College of Agriculture Rewa. The experiment was laid out in CRD design with three replications. The experiment comprised of fifteen treatments consisting of T_1-Control distilled water, T_2-KNO_3 1%, T_3-KNO_3 2%, T_4-Thiourea 1 %, T_5-Thiourea 2 %, T_6-Cow urine 10 %, T_7-Cow urine 20 %, T_8-Cow dung slurry 10 %, T_9-Cow dung slurry 20 %, T_{10}-GA_3 100 ppm, T_{11}-GA_3 200 ppm, T_{12}-NAA 100 ppm, T_{13}-NAA 200 ppm for 24 hours, T_{14}-HCl 0.1 % and T_{15}-H_2SO_4 0.1 % for 2 minutes.

For sowing of custard apple seeds, black polybags (9 cm × 12 cm) were used. To promote drainage and aeration poly bags were punctured and then filled with media, made of three part of soil and one part of FYM. Fully ripe, uniform-sized custard apple fruits were collected and seeds were extracted, washed and dried in the shade for an hour. The seeds were then placed in a bucket of water for whole night. Non-viable floating seeds were discarded, while viable seeds that sank were kept for sowing. The viable extracted seeds were soaked in Pre-sowing treatment solutions prepared by dissolving specific quantities of each substance in 100 ml of distilled water. For KNO_3, 1% and 2% solutions were made by dissolving 1 g and 2 g respectively. Similarly, thiourea solutions of 1% and 2% were prepared using 1 g and 2 g of thiourea. Cow urine treatments at 10% and 20% concentrations were prepared by adding 1 ml and 2 ml of cow urine, respectively. Cow dung slurry solutions of 10% and 20% were made by dissolving 1 g and 2 g of slurry. GA_3 solutions of 100 ppm and 200 ppm were prepared by dissolving 1 mg and 2 mg of GA_3, respectively. NAA solutions of 100 ppm and 200 ppm were similarly prepared using 1 mg and 2 mg of NAA. For acid treatments, 0.1% solutions of HCl and H_2SO_4 were prepared by adding 0.01 ml of each acid to 100 ml of distilled water. All measurements were carried out using an electronic balance to ensure precision and then seed sowing was done on 20-09-2023. For analysis Statistical software use was OPSTAT.

Results and Discussion

1. Seed Germination Parameters

Pre-sowing treatments significantly impacted seed germination parameters represented in table 01. Seeds soaked in GA3 @ 200 ppm for 24 hours had the shortest time to start (21.51 days) and complete germination (49.73 days). The control group (T1) showed the longest times, with 38.97 days to start and 72.36 days to complete germination. The highest germination percentage (78.26%) was in GA3 @ 200 ppm for 24 hours, while the control had the lowest (61.76%). The highest seedling vigour index (769.29) was noted under the chemicals and bio-agents treatment combination of GA_3 @ 200 ppm (T_{11}) Whereas lowest seedling vigour index (459.49) was observed under the treatment of control (T_1). The findings are consistent with those reported by Dhoran and Gudadhe (2012) [7]. The acceleration of specific enzymes such as α-amylase by GA_3 could be responsible for increasing the availability of starch assimilation, leading to early germination. Several researchers have extensively documented the significant impact of GA_3 on seed germination, particularly in overcoming seed dormancy. Garge *et al.* (2011) [9] demonstrated this effect in custard apple, while Krishnan and Kulasekaran (1984) [11], Ghosh and Sen (1988) [10] observed it in ber.

Table 1: Effect of chemicals and bio regulators on Seed germination parameters

Tr. No.	Treatments	Days taken to start germination	Days taken to complete germination	Germination percentage	Seedling Vigour Index
T_1	Control	38.97	72.36	61.76	459.49
T_2	KNO_3 1% 24hours	25.66	59.91	71.66	659.27
T_3	KNO_3 2% 24 hours	24.43	56.24	73.92	691.15
T_4	Thiourea 1% 24 hours	26.13	60.32	70.34	645.01
T_5	Thiourea 2% 24 hours	24.82	57.71	72.46	672.42
T_6	Cow urine 10% 24 hours	28.38	62.80	68.35	612.41
T_7	Cow urine 20% 24 hours	27.55	61.15	69.61	628.57
T_8	Cow dung slurry 10% 24 hours	30.77	64.52	65.96	577.15
T_9	Cow dung slurry 20% 24 hours	30.15	64.96	66.80	589.17
T_{10}	GA_3 100 PPM 24 hours	22.00	50.48	78.08	757.37
T_{11}	GA_3 200 PPM 24 hours	21.51	49.73	78.26	769.29
T_{12}	NAA 100 PPM 24 hours	23.08	55.87	75.65	719.43
T_{13}	NAA 200 PPM 24 hours	22.34	54.02	76.23	726.47
T_{14}	HCl 0.1% 2 Minute	32.90	67.40	62.86	531.79
T_{15}	H_2SO_4 0.1% 2 Minute	32.47	65.87	64.61	554.99
	S.Em ±	0.92	0.97	1.34	5.53
	C.D.	2.69	2.82	3.89	16.05

2. Growth Parameters

Pre-sowing treatments significantly affected growth parameters represented on table 02. Seeds treated with GA_3 at 200 ppm for 24 hours (T_{11}) had the highest plant height, diameter, and no. of leaves at 60, 90 and 120 DAS. Specifically, T_{11} seedlings reached 5.02 cm, 7.25 cm, and 9.83 cm in height, 2.69 mm, 3.21 mm, and 4.08 mm in diameter, and 6.51, 12.18 and 13.71 leaves, respectively. In contrast, the control group (T_1) had the lowest values across all parameters. Ratan and Reddy (2004) have documented an increase in plant height attributed to GA_3. GA_3 fosters plant growth by promoting cell elongation. These findings suggest that applying plant growth regulators to seedlings could be a beneficial approach to enhancing mango seedling

growth, thereby reducing both time and cost in seedling production, as indicated by Mobli *et al.* (2008) [12]. The increased production of leaves in seedlings may be attributed to both enhanced seedling growth and the activity of GA_3 at the apical meristem, resulting in greater synthesis of nucleoprotein responsible for increasing leaf initiation (Sen and Ghunti, 1976) [16].

Table 2: Effect of chemicals and bio regulators on Growth parameters

Tr. No.	Treatments	Height of Seedlings (cm)			Diameter of Seedling (mm)			Leaves per plant		
		60 DAS	90 DAS	120 DAS	60 DAS	90 DAS	120 DAS	60 DAS	90 DAS	120 DAS
T_1	Control	3.36	5.56	7.44	1.48	1.97	2.53	4.21	7.55	8.75
T_2	KNO_3 1% 24 hours	4.34	6.20	9.20	2.28	2.65	3.31	5.97	9.09	10.60
T_3	KNO_3 2% 24 hours	4.41	6.33	9.35	2.35	2.68	3.40	6.16	9.31	10.96
T_4	Thiourea 1% 24 hours	4.22	6.13	9.17	2.21	2.60	3.40	5.59	9.02	10.47
T_5	Thiourea 2% 24 hours	4.36	6.24	9.28	2.29	2.67	3.35	6.03	9.24	10.85
T_6	Cow urine 10% 24 hours	4.02	6.17	8.96	2.11	2.52	3.15	5.36	8.89	10.30
T_7	Cow urine 20% 24 hours	4.16	6.29	9.03	2.16	2.58	3.20	5.43	8.96	10.35
T_8	Cow dung slurry 10% 24 hours	3.81	5.99	8.75	1.90	2.43	3.08	5.16	8.68	10.10
T_9	Cow dung slurry 20% 24 hours	3.94	6.08	8.82	2.08	2.44	3.13	5.29	8.75	10.22
T_{10}	GA_3 100 PPM 24 hours	4.88	7.11	9.70	2.64	3.12	3.89	6.42	11.51	13.42
T_{11}	GA_3 200 PPM 24 hours	5.02	7.25	9.83	2.69	3.21	4.08	6.51	12.18	13.71
T_{12}	NAA 100 PPM 24 hours	4.59	6.46	9.51	2.48	2.73	3.51	6.23	9.37	11.36
T_{13}	NAA 200 PPM 24 hours	4.74	6.69	9.53	2.55	2.76	3.57	6.30	9.49	11.62
T_{14}	HCl 0.1% 2 Minute	3.64	5.82	8.46	1.68	2.17	2.97	4.36	8.15	9.24
T_{15}	H_2SO_4 0.1% 2 Minute	3.78	5.97	8.59	1.83	2.21	3.04	5.03	8.61	9.81
	S.Em ±	0.12	0.07	0.09	0.08	0.04	0.12	0.07	0.33	0.33
	C.D	0.36	0.20	0.26	0.22	0.11	0.35	0.21	0.98	0.96

3. Physiological Parameters

Pre-sowing treatments significantly impacted physiological parameters represented on table 03. Seeds treated with GA_3 at 200 ppm for 24 hours (T_{11}) had the highest fresh weight of leaves (2.83 g), dry weight of leaves (0.25 g), and leaf area index (31.15 cm^2) at 120 DAS. In contrast, the control group (T_1) showed the lowest values: 1.33 g, 0.09 g, and 15.67 cm^2.The accelerated transportation of water and nutrients likely facilitated increased production of photosynthetic products, which were then translocated to different plant parts, potentially leading to enhanced seedling growth, and consequently increased fresh weight. These outcomes are consistent with the findings reported by Dhankhar and Singh (1996) [6] in Aonla and Anjanwe *et al.* (2013) [1] in Papaya. Similar observations to these findings have been documented by Vachhani *et al.* (2014) [19] in khirnee and Ashiya and Tank (2015) [4] in mango.

Table 3: Effect of chemicals and bio regulators on Physiological parameters

Tr. No.	Treatments	Fresh weight of leaves (g)	Dry weight of leaves (g)	Leaf Area Index (cm^2)
T_1	Control	1.33	0.09	15.67
T_2	KNO_3 1% 24 hours	2.47	0.18	25.30
T_3	KNO_3 2% 24 hours	2.51	0.19	26.42
T_4	Thiourea 1% 24 hours	2.44	0.17	24.76
T_5	Thiourea 2% 24 hours	2.48	0.18	26.12
T_6	Cow urine 10% 24 hours	2.48	0.14	23.55
T_7	Cow urine 20% 24 hours	2.42	0.16	24.00
T_8	Cow dung slurry 10% 24 hours	2.34	0.12	22.61
T_9	Cow dung slurry 20% 24 hours	2.37	0.14	22.83
T_{10}	GA_3 100 PPM 24 hours	2.78	0.22	29.10
T_{11}	GA_3 200 PPM 24 hours	2.83	0.25	31.15
T_{12}	NAA 100 PPM 24 hours	2.60	0.20	28.01
T_{13}	NAA 200 PPM 24 hours	2.64	0.21	28.20
T_{14}	HCl 0.1% 2 Minute	2.19	0.11	20.35
T_{15}	H_2SO_4 0.1% 2 Minute	2.23	0.12	21.09
	S.Em ±	0.03	0.02	0.12
	C.D	0.08	0.05	3.55

4. Root Parameters

Pre-sowing treatments significantly impacted root parameters represented on table 04. Seeds treated with GA_3 at 200 ppm for 24 hours (T_{11}) had the highest fresh weight of roots (1.02g), dry weight of roots (0.43g), and length of roots (16.12cm) at 120 DAS. In contrast, the control group (T_1) showed the lowest values: 0.27 g, 0.11 g and 12.70 cm. These results are consistent with the findings reported by Rahemi and Baninasab (2000) [15]. The increased root length resulting from the GA_3 treatment on seeds could be attributed to cell multiplication and elongation in the meristematic region of the roots. These findings align with previous studies by Pampanna and Sulikeri (2001) [13] in Sapota, Dhaka and Pal (2009) [5] in sour lime, Supe *et al.* (2012) [17] in Aonla, Anjanwe *et al.* (2013) [1] in papaya and Parvin *et al.* (2015) [14] in black walnut seeds.

Table 4: Effect of chemicals and bio regulators on root parameters

Tr. No.	Treatments	Fresh weight of root (g)	Dry weight of root (g)	Length of root (cm)
T_1	Control	0.27	0.11	12.70
T_2	KNO_3 1% 24 hours	0.66	0.30	15.03
T_3	KNO_3 2% 24 hours	0.70	0.33	15.15
T_4	Thiourea 1% 24 hours	0.63	0.29	14.88
T_5	Thiourea 2% 24 hours	0.67	0.31	15.10
T_6	Cow urine 10% 24 hours	0.60	0.26	14.39
T_7	Cow urine 20% 24 hours	0.62	0.27	14.41
T_8	Cow dung slurry 10% 24 hours	0.56	0.22	14.22
T_9	Cow dung slurry 20% 24 hours	0.58	0.25	14.24
T_{10}	GA_3 100 PPM 24 hours	0.96	0.42	16.05
T_{11}	GA_3 200 PPM 24 hours	1.02	0.43	16.12
T_{12}	NAA 100 PPM 24 hours	0.79	0.39	15.32
T_{13}	NAA 200 PPM 24 hours	0.82	0.41	15.33
T_{14}	HCl 0.1% 2 Minute	0.36	0.14	13.82
T_{15}	H_2SO_4 0.1% 2 Minute	0.41	0.19	13.90
	S.Em ±	0.03	0.03	0.15
	C.D	0.08	0.09	0.44

5. Economic parameter

B:C ratio was calculated using formula-

B:C Ratio = Gross Returns/Total Costs

The treatment GA_3 at 200 ppm for 24 hours (T_{11}) yielded the highest net return (402.60) and benefit-to-cost (B:C) ratio (2.10), while the control (T_1) showed the lowest net return (188.00) and B:C ratio (0.98). The superior net return and B:C ratio associated with treatment GA_3 at 200 ppm for 24 hours (T_{11}) could be attributed to the increased germination rate and survival of a greater number of plants compared to other treatments. This enhanced germination percentage and seedling establishment may be a result of the positive or synergistic effects of chemicals and bio-regulators on seed germination and survival. Data related to this represented in table 05.

Table 5: Effect of chemicals and bio regulators on benefit cost ratio

Tr. No.	Cost of cultivation	Gross income	Net income	B:C Ratio
T_1	190.00	378	188.00	0.98
T_2	190.96	504	313.96	1.63
T_3	191.92	540	348.08	1.81
T_4	190.10	486	295.90	1.55
T_5	190.20	522	331.90	1.74
T_6	190.01	450	259.99	1.36
T_7	190.02	468	277.98	1.46
T_8	190.01	414	223.99	1.17
T_9	190.02	432	241.98	1.27
T_{10}	190.70	576	383.30	2.00
T_{11}	191.40	594	402.60	2.10
T_{12}	190.60	540	349.40	1.83
T_{13}	191.20	558	366.80	1.91
T_{14}	190.05	378	189.95	0.99
T_{15}	190.05	396	205.95	1.08

Conclusion

Based on the findings of the present investigation, it can be concluded that treatment with GA_3 at a concentration of 200 ppm for 24 hours resulted in early and maximum seed germination, increased height, stem diameter, number of leaves, leaf area, fresh weight and dry weight of leaves and root, and a seedling vigour of seedlings and minimum value is found in control. However, these findings serve as indicators and necessitate further experimentation to establish more consistent and conclusive results before being conveyed to growers.

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