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Effect of gibberellic acid (GA_3) and organic substances on root growth and seedling vigour of Custard Apple (*Annona squamosa* L.) seedlings under shade net condition

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Abstract
The current study, entitled "Effect of gibberellic acid (GA_3) and organic substances on root growth and seedling vigour of Custard Apple (*Annona squamosa* L.) seedlings under shade net condition", was carried out during 2024-2025 at the Horticulture Nursery, Department of Fruit Science, College of Agriculture, IGKV, Raipur (C.G.). The experiment was laid out in a Completely Randomized Design (CRD) with 13 treatments, in three replications. The pre-soaking treatments consisted of GA_3 and various organic substances, detailed as follows:T_0: Control, T_1: Distilled water + 72 hours soaking, T_2: GA_3 @ 400 ppm + 24 hours soaking, T_3: GA_3 @ 500 ppm + 24 hours soaking T_4: *Azospirillum* @ 5% + 24 hours soaking,T_5: *Azospirillum* @ 10% + 24 hours soaking, T_6: PSB @ 5% + 24 hours soaking, T_7: PSB @ 10% + 24 hours soaking, T_8: Cow urine @ 5% + 24 hours soaking, T_9: Cow urine @ 10% + 24 hours soaking, T_{10}: Custard Apple Leaf Extract @ 5% + 24 hours soaking, T_{11}: Custard Apple Leaf Extract @ 10% + 24 hours soaking and T_{12}: Cow dung slurry @ 10% + 24 hours soaking. In conclusion, results of the present investigation showed that among the various pre-soaking treatments, T_2 (GA_3 @ 400 ppm + 24 hours soaking) was the most effective in enhancing root growth and seedling vigour parameters, followed by T_3 (GA_3 @ 500 ppm + 24 hours soaking). Control (T_0) showed the lowest performance across all evaluated parameters. Among the organic treatments, T_9 (Cow urine @ 10% + 24 hours soaking) gave the most favourable results, followed by T_{12} (Cow dung slurry @ 10% + 24 hours soaking). Hence, T_9 may serve as a viable alternative to chemical treatments in organic or low-input production systems.

Keywords: *Azospirillum*, gibberellic acid, cow dung slurry, cow urine, custard apple leaf extract, organic substances, PSB

Introduction

The custard apple (*Annona squamosa* L.) is one of the most delightful tropical and subtropical fruits, primarily cultivated in Asia, Africa and the Americas. It belongs to the Annonaceae family and is known by various names, including sugar apple, sitaphal, sweet sop and sharifa in India. In India, custard apples are the most widely consumed fruit from the Annonaceae family. Maharashtra is the leading producer, followed by Madhya Pradesh, Gujarat, Chhattisgarh, Telangana, Karnataka, Andhra Pradesh, Rajasthan, Kerala and Tamil Nadu (Anonymous, 2022-2023) [2]. The germination of custard apple seeds is inconsistent due to a hard seed coat and dormancy, requiring about 35-50 days to germinate (Setten and Koek-Noorman, 1992) [23]. Research reveals that growth regulators like gibberellic acid (GA_3) can enhance germination percentage (Ratan and Reddy, 2004) [21], pre-treatments with water and organic substances, including cow urine, have also gained attention for their potential to improve germination because they contain nutrients that promote seed vigour (Rajput and Sharma, 2020) [20]. Bioinoculants can also aid in breaking seed dormancy by enhancing nutrient availability and promoting plant growth. Moreover, *Annona squamosa* leaves contain essential minerals (P, K, Fe, Ca, Mg, Na, Cu, Se, Zn) and vitamins (A, B_1, B_2, B_3, B_9, C, E), which may support early seed germination (Kumar *et al.*, 2021) [27]. The primary challenges for custard apple production in India include limited availability of improved planting material and insufficient knowledge about propagation techniques.

Considering the above-mentioned fact, the experiment was carried out to study the impact of gibberellic acid (GA_3) and organic substances on root growth and seedling vigor of custard apple.

Material and Methods

The experiment titled "Effect of gibberellic acid (GA_3) and organic substances on root growth and seedling vigor of Custard Apple (*Annona squamosa* L.) seedlings under shade net condition" was conducted during the period of 2024-2025 at the Horticulture Nursery, Department of Fruit Science, College of Agriculture, IGKV, Raipur (C.G.). A Completely Randomized Design was employed for the study, consisting of 13 treatments with 3 replications. The specific treatments are detailed in Table 1. Gibberellic acid solutions were prepared by dissolving 0.04 g and 0.05 g of GA_3 in a few drops of 95% ethanol or NaOH, followed by the addition of distilled water to reach a final volume of 100 ml, resulting in solutions of 400 ppm and 500 ppm GA_3, respectively. Solutions of *Azospirillum* and Phosphate Solubilizing Bacteria (PSB) were prepared by adding 5 ml and 10 ml of each liquid formulation into separate beakers, then diluting with distilled water to make up 100 ml, producing 5% and 10% solutions, respectively. Similarly, cow urine solutions were made by measuring 5 ml and 10 ml of cow urine into different beakers, followed by dilution with distilled water to total 100 ml, yielding 5% and 10% preparations. A cow dung slurry was created using 10 g of cow dung mixed with distilled water to a final volume of 100 ml, resulting in a 10% slurry. To obtain Custard Apple leaf extract, 10-15 fresh leaves were washed and then crushed with a small amount of distilled water. This extract was diluted by adding 5 ml and 10 ml of leaf extract into separate beakers, followed by the addition of 95 ml and 90 ml of distilled water, producing 5% and 10% Custard Apple leaf extracts, respectively. The pre-soaked seeds were then placed in black polybags filled with a soil, farmyard manure (FYM) and sand mixture in a 2:1:1 ratio at a depth of 5 cm (one seed per bag). The seedlings received light and watering daily until germination, with consistent moisture maintenance and pesticide application to promote healthy seedling development. Observations on growth and seedling vigour parameters were measured at 120 days after sowing. Formulas used to analyse Root: shoot ratio, Seedling Vigour Index- I (SVI- I), Seedling Vigour Index- II (SVI- II) are as follows,

A. Root: shoot ratio = $\dfrac{\text{Dry weight of the root}}{\text{Dry weight of the shoot}}$

B. Seedling Vigour Index- I = Germination percentage x Total length of seedlings at 120 DAS

C. Seedling vigour index - II = Germination percentage × Dry weight of seedling at 120 DAS

Table 1: Details of the Treatment

Sl. No.	Treatment Notations	Treatment Details
1	T_0	Control
2	T_1	Distilled water + 72 hours soaking
3	T_2	GA_3 @ 400 ppm + 24 hours soaking
4	T_3	GA_3 @ 500 ppm + 24 hours soaking
5	T_4	*Azospirillum* @ 5% + 24 hours soaking
6	T_5	*Azospirillum* @ 10% + 24 hours soaking
7	T_6	PSB @ 5% + 24 hours soaking
8	T_7	PSB @ 10% + 24 hours soaking
9	T_8	Cow urine @ 5% + 24 hours soaking
10	T_9	Cow urine @ 10% + 24 hours soaking
11	T_{10}	Custard Apple Leaf Extract @ 5% + 24 hours soaking
12	T_{11}	Custard Apple Leaf Extract @ 10% + 24 hours soaking
13	T_{12}	Cow dung slurry @ 10% + 24 hours soaking

Result and Discussion

The effect of different pre-sowing treatments of GA_3 on root development of seedlings at 120 days after sowing revealed significant variations across treatments. A marked improvement in root growth parameters was recorded under GA_3 application compared to the control. The maximum root length (12.50 cm) was observed in GA_3 @ 400 ppm + 24 hours soaking (T_2), followed by GA_3 @ 500 ppm + 24 hours soaking (T_3), which measured 11.23 cm, whereas the minimum value (6.82 cm) was recorded in the untreated control (T_0). Similarly, the highest number of primary roots (21.34) and secondary roots (46.08) were noted in T_2, followed by T_3, which showed 19.77 and 40.34, respectively, while the control produced the lowest counts (13.68 and 27.53, respectively). Fresh root weight was maximum in T_2 (1.33 g), followed by T_3 (1.02 g), whereas the control recorded only 0.63 g. Likewise, dry root weight followed the same trend, with T_2 producing the highest value (0.67 g), followed by T_3 (0.58 g), while the lowest dry root weight (0.26 g) was observed in the control, followed by T_1 (0.35 g). The enhancement in root length, root number and root biomass can be attributed to the multifaceted role of gibberellic acid in improving seed germination and seedling vigour by activating gluconeogenic enzymes, enhancing nutrient uptake and mobilization, stimulating cell elongation and division, regulating auxin metabolism and improving cell wall flexibility and membrane permeability, which together promoted root elongation and biomass accumulation. Similar beneficial effects of GA_3 on root length and vigour have been reported by Parmar *et al.* (2016) [16], Palepad *et al.* (2017) [14], Yadav *et al.* (2018) [25], Rajput and Sharma (2020) [20] and Sunder *et al.* (2024) [24]. Enhanced primary and secondary root development under GA_3 treatments corroborates the findings of Yadav *et al.* (2018) [25], Mane *et al.* (2019) [11] and Hazarika *et al.* (2023) [6]. The positive response in secondary root proliferation is also in line with the reports of Wagh *et al.* (1998) [28] and Palanisamy and Ramamoorthy (1987) [13], as well as

Anburani and Shakila (2010) [1] in other crops. Improvements in fresh and dry root biomass due to GA_3 agree with the observations of Gurung *et al.* (2014) [5], Patel *et al.* (2012) [17], Rahangdale *et al.* (2019) [19], Mane *et al.* (2019) [11] and Rajput and Sharma (2020) [20]. Collectively, these findings confirm that GA_3 at moderate concentration (400 ppm) with optimal soaking duration significantly improves root system architecture and biomass accumulation.

The effect of GA_3 treatments on shoot growth, biomass allocation and seedling vigour showed significant improvement over the control at 120 days after sowing. The maximum fresh shoot weight (3.84 g) was recorded with GA_3 @ 400 ppm + 24 hours soaking (T_2), followed by GA3 @ 500 ppm + 24 hours soaking (T_3), which measured 3.62 g, whereas the minimum fresh shoot weight (1.59 g) was observed in the untreated control (T_0). A similar trend was noted for dry shoot weight, which was highest in T_2 (1.68 g), followed by T_3 (1.53 g), while the lowest value (0.53 g) was recorded in T_0. The enhancement in both fresh and dry shoot biomass can be attributed to the regulatory role of gibberellic acid in promoting cell elongation, cell division and the translocation of assimilates, resulting in improved dry matter accumulation in different plant parts. These findings are consistent with those reported by Meena and Jain (2012) [29], Yadav *et al.* (2018) [25], Mane *et al.* (2018) [30], Rahangdale *et al.* (2019) [19], Rajput and Sharma (2020) [20], Lawhale *et al.* (2020) [9] and Panherkar *et al.* (2021) [15]. The root-to-shoot ratio also exhibited a similar response, with the highest ratio (0.43) recorded in T_2, followed by T_3 (0.38), while the lowest ratio (0.23) was observed in T_0. The improved ratio reflects a balanced enhancement in both root and shoot dry weights under GA_3 application, which could be due to enhanced cell division and elongation, leading to better overall plant growth. Similar improvements in root: shoot balance under growth regulator treatments were reported by Misra and Jaiswal (2001) [31], Malti *et al.* (2003) [10], Zhao *et al.* (2004) [26], Lalitha *et al.* (2020) [8] and Dhorajiya *et al.* (2022) [4]. Seedling Vigour Indices were also markedly enhanced by GA_3 application. The highest Seedling Vigour Index-I (2225.96) was recorded in T_2, followed by T_3 (2016.79), while the lowest value (861.63) was noted in the control. Likewise, Seedling Vigour Index-II was maximum in T_2 (193.24), followed by T_3 (166.88) and minimum in T_0 (41.17). The improvement in seedling vigour indices can be attributed to GA_3 mediated enhancement in germination percentage, shoot and root lengths and dry biomass accumulation, which improve the assimilation and distribution of nutrients within the seedlings (Brain and Hemming, 1955). These observations are in close agreement with the reports of Kumar *et al.* (2011) [7], Padma *et al.* (2013) [12], Gurung *et al.* (2014) [5], Palepad *et al.* (2017) [14], Lawhale *et al.* (2020) [9], Rajput and Sharma (2020) [20], Sen *et al.* (2003) [22], Patil *et al.* (2012) [18], Parmar *et al.* (2016) [16], Jain *et al.* (2017) [32] and Hazarika *et al.* (2023). Overall, GA_3 @ 400 ppm with 24 hours soaking consistently produced the best results, indicating that an optimal concentration of GA_3 effectively enhances shoot biomass, root-shoot ratio and seedling vigour.

Table 2: Effect of GA_3 and organic substances on root growth parameters of custard apple seedlings

Treatment notations	Treatment details	Root length (cm) at 120 DAS	Number of primary roots at 120 DAS	Number of secondary roots at 120 DAS	Fresh root weight (g) at 120 DAS	Dry root weight (g) at 120 DAS
T_0	Control	6.82	13.68	27.53	0.63	0.26
T_1	Distilled water + 72 hours soaking	7.73	15.42	29.70	0.75	0.35
T_2	GA_3 @ 400 ppm + 24 hours soaking	12.50	21.34	46.08	1.33	0.67
T_3	GA_3 @ 500 ppm + 24 hours soaking	11.23	19.77	40.34	1.02	0.58
T_4	*Azospirillum* @ 5% + 24 hours soaking	9.60	16.75	33.43	0.86	0.47
T_5	*Azospirillum* @ 10% + 24 hours soaking	9.84	17.87	35.42	0.95	0.53
T_6	PSB @ 5% + 24 hours soaking	9.47	16.56	32.25	0.83	0.45
T_7	PSB @ 10% + 24 hours soaking	9.76	17.41	35.20	0.91	0.51
T_8	Cow urine @ 5% + 24 hours soaking	9.66	17.15	34.67	0.89	0.49
T_9	Cow urine @ 10% + 24 hours soaking	11.02	19.25	37.54	0.97	0.56
T_10	Custard Apple Leaf Extract @ 5% + 24 hours soaking	8.08	16.18	29.49	0.77	0.37
T_{11}	Custard Apple Leaf Extract @ 10% + 24 hours soaking	8.17	16.36	31.20	0.80	0.39
T_{12}	Cow dung slurry @ 10% + 24 hours soaking	10.16	18.14	36.63	0.96	0.54
	SE (m) ±	0.09	0.24	0.61	0.01	0.01
	C.D. at 5%	0.27	0.70	1.77	0.03	0.02

Table 3: Effect of GA_3 and organic substances on seedling vigour of custard apple seedlings

Treatment notations	Treatment details	Fresh shoot weight (g) at 120 DAS	Dry shoot weight (g) at 120 DAS	Root: shoot ratio at 120 DAS	SVI - I at 120 DAS	SVI - II at 120 DAS
T_0	Control	1.59	0.53	0.23	861.63	41.17
T_1	Distilled water + 72 hours soaking	2.29	0.78	0.25	1428.81	77.33
T_2	GA_3 @ 400 ppm + 24 hours soaking	3.84	1.68	0.43	2225.96	193.24
T_3	GA_3 @ 500 ppm + 24 hours soaking	3.62	1.53	0.38	2016.79	166.88
T_4	*Azospirillum* @ 5% + 24 hours soaking	2.77	1.15	0.29	1676.72	117.38
T_5	*Azospirillum* @ 10% + 24 hours soaking	3.28	1.27	0.34	1767.12	133.20
T_6	PSB @ 5% + 24 hours soaking	2.69	1.10	0.28	1636.11	110.89
T_7	PSB @ 10% + 24 hours soaking	3.16	1.24	0.31	1743.76	128.38
T_8	Cow urine @ 5% + 24 hours soaking	2.93	1.20	0.30	1709.66	123.37
T_9	Cow urine @ 10% + 24 hours soaking	3.46	1.34	0.35	1974.49	148.69
$T_1$0	Custard Apple Leaf Extract @ 5% + 24 hours soaking	2.46	1.06	0.27	1473.04	98.76
T_{11}	Custard Apple Leaf Extract @ 10% + 24 hours soaking	2.57	1.08	0.27	1529.83	104.50
T_{12}	Cow dung slurry @ 10% + 24 hours soaking	3.33	1.30	0.34	1825.58	138.40
	SE (m) ±	0.04	0.01	0.01	27.27	1.54
	C.D. at 5%	0.12	0.03	0.02	79.28	4.48

Conclusion

The study clearly demonstrated that pre-sowing seed treatment with GA3 @ 400 ppm + 24 hours soaking (T_2) significantly enhanced root length, number of primary and secondary roots, fresh and dry root weights, fresh and dry shoot weights, root: shoot ratio and seedling vigour indices (SVI-I and SVI-II) in custard apple seedlings. Among organic treatments, cow urine @ 10% + 24 hours soaking (T_9) proved most effective, followed by cow dung slurry @ 10% (T_{12}) and Azospirillum @ 10% (T_5), indicating their potential as eco-friendly alternatives where chemical treatments are not preferred. Overall, GA3 application at 400 ppm emerged as the most efficient treatment for improving seedling growth and vigour, while selected organic treatments also showed promising results for sustainable nursery practices in custard apple propagation.

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