**Effect of gibberellic acid (GA3) and organic substances on seed germination and shoot growth of Custard Apple (*Annona squamosa* L.) seedlings under shade net condition**

**Abstract**

The current study, was conducted during 2024-2025 at Horticulture nursery, Department of Fruit Science, College of Agriculture, IGKV, Raipur (C.G.). The experimental design utilized was Completely Randomized Design, comprising 13 treatments, each replicated three times. The soaking agents included GA3 and organic substances with specific treatments as follows: T0: Control, T1: Distilled water + 72 hours soaking, T2: GA3 @ 400 ppm + 24 hours soaking, T3: GA3 @ 500 ppm + 24 hours soaking T4: *Azospirillum* @ 5% + 24 hours soaking,T5: *Azospirillum* @ 10% + 24 hours soaking, T6: PSB @ 5% + 24 hours soaking, T7: PSB @ 10% + 24 hours soaking, T8: Cow urine @ 5% + 24 hours soaking, T9: Cow urine @ 10% + 24 hours soaking, T10: Custard Apple Leaf Extract @ 5% + 24 hours soaking, T11: Custard Apple Leaf Extract @ 10% + 24 hours soaking and T12: Cow dung slurry @ 10% + 24 hours soaking. The results of the present investigation showed that among the various pre-soaking treatments, T2 (GA3 @ 400 ppm + 24 hours soaking) was the most effective in enhancing seed germination and shoot growth parameters, followed by T3 (GA3 @ 500 ppm + 24 hours soaking). In contrast, the control treatment (T0) exhibited the lowest performance across all evaluated parameters. Among the organic treatments, T9 (Cow urine @ 10% + 24 hours soaking) produced the most favourable response, followed by T12 (Cow dung slurry @ 10% + 24 hours soaking). In conclusion, T9 may serve as a viable alternative to chemical treatments in organic or low-input production systems.

**Key words:** *Azospirillum*, Gibberellic acid, Cow dung slurry, Cow urine, Custard Apple Leaf Extract, Organic substances, PSB.

**Introduction**

Custard Apple (*Annona squamosa* L.) is a highly valued tropical and subtropical fruit crop cultivated primarily in Asia, Africa, and the Americas. Belonging to the family Annonaceae, it is commonly known in India as sitaphal, sugar apple, sharifa, or sweetsop. Maharashtra is the leading producer, followed by Madhya Pradesh, Gujarat, Chhattisgarh, Telangana, Karnataka, Andhra Pradesh, Rajasthan, Kerala and Tamil Nadu (Anonymous, 2022–2023). Custard apple seeds typically require 35–50 days to germinate (Setten and Koek-Noorman, 1992) and germination is often irregular due to dormancy or a hard seed coat. Limited research in India and elsewhere has demonstrated that pre-sowing treatments with GA₃ (150–500 ppm) and other agents can enhance germination (Banker, 1987; Pawashe, 1997; Stino, 1996; Ratan and Reddy, 2004). Pre-treatment of seeds with water, chemical or organic substances is essential for improving germination percentage and seedling vigor. Gibberellic acid (GA₃) facilitates germination by mobilizing endosperm reserves, weakening the endosperm barrier and stimulating embryo growth (Ratan and Reddy, 2004). Due to the increasing cost of synthetic growth regulators, interest has shifted toward accessible, low-cost organic alternatives. Organic substances such as cow dung and cow urine are now gaining attention for their potential to break seed dormancy (Rajput and Sharma, 2020). Additionally, bioinoculants like *Aspergillum* and phosphate-solubilizing bacteria (PSB) can promote germination through the production of plant growth-promoting substances. Moreover, *Annona squamosa* leaves contain essential minerals (P, K, Fe, Ca, Mg, Na, Cu, Se, Zn) and vitamins (A, B₁, B₂, B₃, B₉, C, E), which may support early seed germination (Kumar *et al.,* 2021). The objective of the research is to study the impact of GA3 and organic substances on seed germination and shoot growth of custard apple.

**Materials and Method**

 The present experiment, entitled “Effect of gibberellic acid (GA3) and organic substances on seed germination and shoot growth of Custard Apple (*Annona squamosa* L.) seedlings under shade net condition”, was conducted during 2024-2025 at Horticulture nursery, Department of Fruit Science, College of Agriculture, IGKV, Raipur (C.G.). The experimental design utilized was Completely Randomized Design, 13 treatments, 3 replications and total number of seedlings per treatment is 45. The treatment details are mentioned in table 1. GA₃ solutions are prepared by dissolving (0.04 g) and (0.05 g) GA3 in few drops of 95% ethanol or NaOH and distilled water is added to make up 100 ml solution, yielding 400 ppm and 500 ppm GA3 solutions. *Azospirillum* and PSB solutions are prepared by measuring 5 ml and 10 ml of each liquid formulations into separate beakers then, 95 ml and 90 ml distilled water is added to make up 100 ml solution, yielding 5% and 10% solutions of *Azospirillum* and PSB, respectively. Cow urine solutions are prepared by measuring 5 ml and 10 ml of cow urine into separate beakers then, 95 ml and 90 ml distilled water is added to make up 100 ml solution, yielding 5% and 10% cow urine solution. Cow Dung Slurry is prepared using 10 g of cow dung and distilled water is added to make up 100 ml solution, giving 10% cow dung slurry. To prepare Custard Apple Leaf Extract, 10-15 green leaves are washed, crush/grind with little distilled water to obtain the extract, to measured 5 ml and 10 ml leaf extract add 95 ml and 90 ml distilled water, yielding 5% and 10% Custard Apple Leaf Extract respectively. The pre-soaked seeds were placed in black poly bags filled with soil, FYM and sand in the ratio 2:1:1, at a depth of 5 cm (one seed per bag) and they were lightly watered daily until germination, with regular watering maintained for the water table and pesticide applied for healthy seedlings, observations on germination parameters were recorded daily and growth parameters recorded after 30, 60, 90 and 120 days after sowing. Formulas used to analyse A. Germination percentage (%); B. Survival percentage (%); C. Germination Index (GI) are as follows,



A.



B.



C.

**Fig 1: Formulas to analyse germination parameters**

|  |  |  |
| --- | --- | --- |
| **S.l. No.** | **Treatment Notations** | **Treatment Details** |
| 1 | T0 | Control |
| 2 | T1 | Distilled water + 72 hours soaking |
| 3 | T2 | GA3 @ 400 ppm + 24 hours soaking |
| 4 | T3 | GA3 @ 500 ppm + 24 hours soaking |
| 5 | T4 | *Azospirillum* @ 5% + 24 hours soaking |
| 6 | T5 | *Azospirillum* @ 10% + 24 hours soaking |
| 7 | T6 | PSB @ 5% + 24 hours soaking |
| 8 | T7 | PSB @ 10% + 24 hours soaking |
| 9 | T8 | Cow urine @ 5% + 24 hours soaking |
| 10 | T9 | Cow urine @ 10% + 24 hours soaking |
| 11 | T10 | Custard Apple Leaf Extract @ 5% + 24 hours soaking |
| 12 | T11 | Custard Apple Leaf Extract @ 10% + 24 hours soaking |
| 13 | T12 | Cow dung slurry @ 10% + 24 hours soaking |

**Result and Discussion**

**A: Seed germination parameters**

The results in table 2, indicated that seeds treated with T2 (GA3 @ 400 ppm + 24 hours soaking) exhibited the most rapid germination, initiating at 19.09 days, followed by T3 (GA3 @ 500 ppm + 24 hours soaking) at 20.29 days. In contrast, T0 (Control) recorded the highest number of days to germination (26.29 days). These findings align closely with those reported by Singh *et al.* (2023)*.* This improvement in germination time is the GA₃ role in stimulating enzyme production and activating food reserve mobilization, both of which are critical for initiating early germination (Heden *et al*., 2012).

The shortest time to achieve 50% germination (28.06 days) was recorded under treatment T2 (GA3 @ 400 ppm + 24 hours soaking), followed by T3 (GA3 @ 500 ppm + 24 hours soaking) at 33.56 days (Table 2). The longest duration was observed in T0 (Control), taking 45.57 days. These findings align closely with those reported by Babu *et al.* (2010); Martinez *et al.* (2016); Singh *et al.* (2023)*.* The enhanced germination response is the GA3 role in stimulating α-amylase activity, which facilitates the hydrolysis of starch into simpler sugars, thereby releasing energy required for the activation of embryonic cells (Anjanawe *et al*., 2013).

**Table 1: Details of the Treatment**

The highest germination percentage was recorded in T2 (GA3 @ 400 ppm + 24 hours soaking) 82.23%, which was statistically *at par* with T3 (GA3 @ 500 ppm + 24 hours soaking) 79.09% (Table 2). The lowest germination percentage was observed in T0 (Control), 52.24%. These findings align closely with those reported by Barche *et al.* (2010); Deb *et al.* (2010); Garge *et al.* (2011); Yadav *et al.* (2018); Panherkar *et al.* (2021). The increase in germination percentage is the GA₃ induced stimulation of α-amylase activity, which promotes starch hydrolysis into simple sugars, providing the energy required for embryo activation.

In terms of survival percentage, T2 (GA3 @ 400 ppm + 24 hours soaking) recorded the highest value 84.43%, followed by T3 (GA3 @ 500 ppm + 24 hours soaking) 80.47%, while T0 (Control) showed the lowest survival rates 67.07% (Table 2). These findings align closely with those reported by Parmar *et al.* (2016); Palepad *et al.* (2017); Patel *et al.* (2017); Panherkar *et al.* (2021); Lawhale *et al.* (2020). The improved survival rate is the GA₃ role in increasing seedling dry weight through enhanced cell elongation and expansion, resulting in better root and shoot development (Madgaonkar, 2013).

The germination index was also maximum in T2 (GA3 @ 400 ppm + 24 hours soaking) 3.40, followed by T3 (GA3 @ 500 ppm + 24 hours soaking) 2.84, whereas the lowest germination index was recorded in T0 (Control) 1.47 (Table 2). These findings align closely with those reported by Palepad *et al.* (2017); Patel *et al.* (2017); Hazarika *et al.* (2023). The enhancement in germination index is the role of GA₃ in initiating enzyme production and activating food reserve mobilization, both of which are essential for promoting early and uniform germination (Heden *et al.,* 2012).

**B: Shoot growth parameters**

 The results in table 3, indicated that maximum seedling height at 30, 60, 90 and 120 days after sowing were recorded in T2 (GA3 @ 400 ppm + 24 hours soaking) with 7.01, 8.57, 11.07 and 14.57 cm, respectively, while the lowest values were observed in the T0 (Control) with 3.21, 4.71, 7.21 and 9.68 cm. These findings align closely with those reported by Yadav *et al.* (2018); Rahangdale *et al.* (2019); Rajput and Sharma (2020); Rana *et al.* (2020); Sunder *et al.* (2024). This increase in plant height is the role of GA₃ at various stages, as it promotes internode elongation and is known to enhance cell elongation (Heden *et al.*, 2012).

The highest number of leaves (6.74, 8.30, 11.54 and 12.54) at 30, 60, 90 and 120 DAS, respectively, was recorded in T2 (GA3 @ 400 ppm + 24 hours soaking), while the lowest (2.44, 3.83, 6.30 and 7.30) was observed in the T0 (Control) (Table 3). These findings align closely with those reported by Mane *et. al* (2018); Yadav *et al.* (2018); Rana *et al.* (2020). This The increase in leaf number is the GA₃ activity in apical meristem, which enhances nucleoprotein synthesis and promotes leaf initiation (Heden *et al.,* 2012).

The maximum collar diameter (1.91, 2.65, 3.40 and 3.70 mm) at 30, 60, 90 and 120 DAS was recorded in T2 (GA3 @ 400 ppm + 24 hours soaking), while the minimum (0.78, 1.44, 2.19 and 2.49 mm) was observed in the T0 (Control) (Table 3). These findings align closely with those reported by Rahangdale *et al.* (2019); Rajput and Sharma (2020). The increase in collar diameter is GA₃ induced stimulation of cell division and elongation in stem tissues, likely through enhanced cambial activity (Dhankar and Singh, 1996).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Treatment notations** | **Treatment details** | **Days taken to initiate germination** | **Days taken for 50% germination** | **Germination %** | **Survival %** | **Germination Index** |
| **T0** | Control | 26.29 | 45.57 |  52.24 | 67.07 | 1.47 |
| **T1** | Distilled water + 72 hours soaking |  24.98 |  43.55 |  68.43 |  71.44 |  2.16 |
| **T2** | GA3 @ 400 ppm + 24 hours soaking | 19.09 | 28.06 |  82.23 | 84.43 | 3.40 |
| **T3** | GA3 @ 500 ppm + 24 hours soaking | 20.29 | 33.56 | 79.09 | 80.47 | 2.84 |
| **T4** | *Azospirillum* @ 5% + 24 hours soaking |  22.74 |  40.27 |  72.46 |  74.42 |  2.41 |
| **T5** | *Azospirillum* @ 10% + 24 hours soaking |  21.65 | 39.04 | 74.00 | 78.04 | 2.60 |
| **T6** | PSB @ 5% + 24 hours soaking |  22.96 |  41.08 |  71.54 | 74.22 | 2.35 |
| **T7** | PSB @ 10% + 24 hours soaking |  22.24 | 39.26 |  73.36 | 76.71 | 2.52 |
| **T8** | Cow urine @ 5% + 24 hours soaking |  22.56 |  40.03 | 73.00 | 75.60 |  2.46 |
| **T9** | Cow urine @ 10% + 24 hours soaking |  20.85 |  37.11 |  78.26 | 79.21 |  2.81 |
| **T10** | Custard Apple Leaf Extract @ 5% + 24 hours soaking |  23.55 |  42.23 |  69.06 |  72.74 |  2.21 |
| **T11** | Custard Apple Leaf Extract @ 10% + 24 hours soaking |  23.23 |  42.02 |  71.09 |  72.83 |  2.31 |
| **T12** | Cow dung slurry @ 10% + 24 hours soaking |  21.39 |  38.23 |  75.22 |  78.16 |  2.68 |
|  | **SE (m) ±** | **0.28** | **0.58** | **1.26** | **1.03** | **0.03** |
| **C.D. at 5%** | **0.81** | **1.68** | **3.67** | **2.98** | **0.09** |

**Table 2: Effect of GA3 and organic substances on seed germination parameters of custard apple seedlings**

**Table 3: Effect of GA3 and organic substances on shoot growth parameters of custard apple seedlings**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Treatment notations** |  **Treatment details** | **Seedling height (cm)** | **Number of leaves** |  **Collar diameter (mm)** |
| **30 DAS** | **60 DAS** | **90 DAS** | **120 DAS** | **30 DAS** | **60 DAS** | **90 DAS** | **120 DAS** | **30 DAS** | **60 DAS** | **90 DAS** | **120 DAS** |
| **T0** |  Control | 3.21 | 4.71 | 7.21 | 9.68 | 2.44 | 3.83 | 6.30 | 7.30 | 0.78 | 1.44 |  2.19 |  2.49 |
| **T1** |  Distilled water + 72 hours soaking | 5.50 | 7.15 | 9.65 | 12.43 | 4.03 | 6.02 | 8.05 | 9.04 | 1.19 | 2.06 |  2.81 |  3.11 |
| **T2** |  GA3 @ 400 ppm + 24 hours soaking | 7.01 | 8.57 | 11.07 | 14.57 |  6.74 | 8.30 | 11.54 | 12.54 | 1.91 | 2.65 |  3.40 | 3.70 |
| **T3** |  GA3 @ 500 ppm + 24 hours soaking | 6.61 | 8.28 | 10.78 | 14.28 |  6.35 |  7.65 | 10.70 | 11.71 | 1.78 | 2.52 |  3.27 |  3.57 |
| **T4** |  *Azospirillum* @ 5% + 24 hours soaking | 5.87 | 7.54 | 10.04 | 13.54 |  4.73 |  7.09 | 8.73 | 9.72 | 1.52 |  2.28 |  3.03 |  3.33 |
| **T5** |  *Azospirillum* @ 10% + 24 hours soaking | 6.34 | 8.04 | 10.51 | 14.04 |  5.43 |  7.23 | 9.62 | 10.54 | 1.63 |  2.38 |  3.11 |  3.41 |
| **T6** |  PSB @ 5% + 24 hours soaking | 5.73 | 7.49 | 10.00 | 13.40 |  4.55 |  6.81 | 8.55 | 9.54 | 1.46 |  2.27 |  3.02 |  3.32 |
| **T7** |  PSB @ 10% + 24 hours soaking | 6.27 | 8.01 | 10.44 | 14.01 |  5.32 |  7.20 | 9.34 | 10.35 | 1.59 | 2.34 |  3.09 |  3.39 |
| **T8** |  Cow urine @ 5% + 24 hours soaking | 6.10 | 7.76 | 10.26 | 13.76 |  5.13 |  7.11 | 9.15 | 10.15 | 1.55 | 2.30 |  3.05 |  3.35 |
| **T9** |  Cow urine @ 10% + 24 hours soaking | 6.54 | 8.21 | 10.71 | 14.21 |  6.09 | 7.41 | 10.53 | 11.52 | 1.72 | 2.46 |  3.21 |  3.51 |
| **T10** |  Custard Apple Leaf Extract @ 5% + 24 hours soaking | 5.60 | 7.25 | 9.75 | 13.23 | 4.15 |  6.22 | 8.14 | 9.13 | 1.32 | 2.16 |  2.91 |  3.21 |
| **T11** |  Custard Apple Leaf Extract @ 10% + 24 hours soaking | 5.70 | 7.35 | 9.83 | 13.35 |  4.51 |  6.62 | 8.55 | 9.33 | 1.30 |  2.26 |  3.01 |  3.31 |
| **T12** |  Cow dung slurry @ 10% + 24 hours soaking | 6.44 | 8.11 | 10.61 | 14.11 |  5.76 |  7.23 | 9.72 | 10.73 | 1.65 | 2.38 |  3.13 |  3.43 |
|  | **SE (m) ±** | **0.09** | **0.09** | **0.15** | **0.18** | **0.09** | **0.09** | **0.15** | **0.18** | **0.03** | **0.04** | **0.05** | **0.06** |
| **C.D. at 5%** | **0.27** | **0.27** | **0.43** | **0.53** | **0.25** | **0.27** | **0.44** | **0.54** | **0.08** | **0.10** | **0.14** | **0.16** |

**Conclusion**

The application of GA3 @ 400 ppm + 24 hours soaking (T2) significantly improved germination by reducing the time to initiation and 50% germination, while enhancing germination percentage, survival rate, germination index, seedling height, number of leaves and collar diameter in custard apple seedlings. Among organic treatments, T9 (Cow urine @ 10% + 24 hours soaking) proved most effective, followed by T12 (Cow dung slurry @ 10% + 24 hours soaking) and T5 (*Azospirillum* @ 10% + 24 hours soaking), indicating their potential as suitable alternatives where chemical use is not preferred.

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