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Effect of GA_3, NAA and Biofertilizers on seed germination and seedling growth of custard apple

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Abstract
The present experiment entitled, "Effect of GA3, NAA and Biofertilizers on seed germination and seedling growth of custard apple" was designed with an objective to study the effect of different concentration of PGR and biofertilizers on seed germination and seedling growth of custard apple. The experiment was conducted at the student instructional field and Shed-net House, Department of Horticulture, School of Agricultural Sciences, G. H. Raisoni University Saikheda, Dist.- Pandhurna (M.P.) during *Rabi* Season of Year 2024. The experiment was laid out in RBD (Randomized Block Design) with 11 treatments including control, the detail of experiment are, T_1- Control, T_2- Gibberellic Acid @ 50%, T_3- Gibberellic Acid @ 75%, T_4- Gibberellic Acid @ 100%, T_5- Naphthalene Acetic Acid @ 50%, T_6- Naphthalene Acetic Acid @ 75%, T_7- Naphthalene Acetic Acid @ 100%, T_8- Azatobactor (5 ml/l of water), T_9- Azatobactor (10 ml/l of water), T_{10}- Azospirillum (5 ml/l of water) and T_{11}- Azospirillum (10 ml/l of water). The observation on growth characters was recorded under the experiments is days to germination (Days), days to 50% germination (Days), days to 100% germination (Days), height of seedling at 60 DAS, height of seedling (cm) 90 DAS, diameter of seedling (cm) at 60 DAS, diameter of seedling at 90 DAS, fresh weight of shoot at 60 DAS, dry weight of shoot (g) at 60 DAS, fresh weight of shoot at 90 DAS and dry weight of shoot (g) at 90 DAS. The result revealed that the treatment T4 (Gibberellic Acid @ 100 ppm) consistently outperformed all other treatments, producing the earliest seed germination (28.43 days), 50% germination (33.19 days), and full germination (38.30 days), whereas the control (T1) exhibited the most delayed germination (36.02, 40.51, and 47.02 days. Seedling growth parameters were also markedly improved under T4. The maximum height was achieved under this treatment (15.25 cm at 60 DAS and 30.70 cm at 90 DAS), along with the greatest seedling diameter (0.356 cm and 0.515 cm. This treatment further resulted in the highest fresh shoot weights (1.73 g and 2.66 g at 60 and 90 DAS) and dry shoot weights (0.170 g and 0.513 g). Treatments T6 (NAA @ 75 ppm) and T3 (GA_3 @ 75 ppm) followed closely and performed statistically at par with T4 in several parameters, while the control recorded the lowest values across all traits.

Keywords: GA3, NAA, biofertilizers, seed germination and custard apple

Introduction
Custard apple (*Annona squamosa* L.), is a fruit crop that originated in Central America and spread to Mexico and Tropical America. It contain chromosome number (2n=14), a member of the Annonaceae family, is one of the best fruit that tropical America has provided to India. There are over 100 species in the genus, five of which produce edible fruits. The fresh fruit contains approximately 28-55% of edible portion consisting of 73.30% moisture, 1.60% protein, 0.30% fat, 0.70% mineral matter, 23.90% carbohydrates, 0.20% calcium, 0.40% phosphorus, 1.0% iron, 12.4-18.15% sugar and 0.26-0.65% acidity with caloric value of 105 K Cal/100g. (Jain *et al.,* 2017) [6]. Irregular germination, in Custard apple seeds may be due to dormancy or due to hard seed coat. The utility of GA3 from 150-500 ppm is helpful for getting better germination of custard apple seeds (Ratan and Reddy, 2004) [10]. As a result, pre-treating custard apple seeds with various organics and chemicals is critical in order to maximize germination. The use of plant growth regulators in proper concentration with scarification may regulate seed germination and seedling growth behaviour in many fruit crops. Seed requires pre-treatments prior to sowing in order to achieve higher and proper germination with vigorous seedling development.

Treating seeds with GA3 also helps enhancing their growth (Chadha, 2010) [5]. For enhancing seed germination and growth of seedling GA3 was used. With the passage of time, old seeds lose their capacity to germinate. Using germination promoters, however, it is possible to extend seed germination capacity (Parmar *et al.,* 2016) [8]. Pre-sowing treatment of growth regulators could lead to increase seed germination and enhancement of seedling growth. Plant growth regulators like GA3 enhance the germination, growth and survival of seedlings. GA3 are used for weakening of the seed coat so that the emergence of radical and plumule is positively influenced for root and shoot formation (Rahangdale *et al.,* 2019) [11].

The effect of NAA on plant growth is greatly dependent on the time of admission and concentration. NAA has been shown to greatly increase cellulose fibre formation in plants. (Mahaveer *et al.,* 2017) [7]. Biomix is the well balanced and a unique blend of selected species of microbes which fix atmospheric nitrogen in the soil making it available to plants. It also contain microbes which can solubilized residual Phosphate, Iron, and Magnesium etc. from soil making the more easily available to the plants. It stimulates sprouting and help to increase the water holding capacity of the soil. It playa multifunctional role: As vital components of organic amendments and composts, as a mean of suppressing insects and plant diseases, to improve crop quality and yields, restore soil microflora. Increase seed germination, promote deep rooting system, solubilize and remove phosphate residual from soil, enhance nutrients cycling. Biomix improve growth, yield and quality of crops adding to farm probability (Syed Shabnam, 2021) [15].

Materials and Methods

The present experiment entitled, "Effect of GA3, NAA and Biofertilizers on seed germination and seedling growth of custard apple" was designed with an objective to study the effect of different concentration of PGR and biofertilizers on seed germination and seedling growth of custard apple. The experiment was conducted at the student instructional field and Shed-net House, Department of Horticulture, School of Agricultural Sciences, G. H. Raisoni University Saikheda, Dist.-Pandhurna (M.P.) during *Rabi* Season of Year 2024. The experiment was laid out in RBD (Randomized Block Design) with 11 treatments including control, the detail of experiment are, T_1- Control, T_2- Gibberellic Acid @ 50%, T_3- Gibberellic Acid @ 75%, T_4- Gibberellic Acid @ 100%, T_5- Naphthalene Acetic Acid @ 50%, T_6- Naphthalene Acetic Acid @ 75%, T_7- Naphthalene Acetic Acid @ 100%, T_8- Azatobactor (5 ml/l of water), T_9- Azatobactor (10 ml/l of water), T_{10}- Azospirillum (5 ml/l of water) and T_{11}- Azospirillum (10 ml/l of water). The observation on growth characters was recorded under the experiments is days to germination (Days), days to 50% germination (Days), days to 100% germination (Days), height of seedling at 60 DAS, height of seedling (cm) 90 DAS, diameter of seedling (cm) at 60 DAS, diameter of seedling at 90 DAS, fresh weight of shoot at 60 DAS, dry weight of shoot (g) at 60 DAS, fresh weight of shoot at 90 DAS and dry weight of shoot (g) at 90 DAS.

- **Days to Germination (Days):** Number of days taken from sowing to the appearance of the first visible germinated seedling.
- **Days to 50% Germination (Days):** Number of days taken for 50% of the total sown seeds to germinate in each treatment.
- **Height of Seedling (cm):** Measured from the base (soil surface) to the tip of the apical meristem using a standard scale or ruler.
- **Diameter of Seedling (cm):** Diameter of the seedling stem measured at the collar region (just above the soil surface), using a digital caliper.

Result and Discussion

Days to Germination (Days)

The earliest seed germination, 50% germination, and 100% germination were observed under the treatment T4 (Gibberellic Acid @ 100 ppm) with values of 28.43 days, 33.19 days, and 38.30 days, respectively. This was followed by T6 (Naphthalene Acetic Acid @ 75 ppm) and T3 (Gibberellic Acid @ 75 ppm), while the maximum (i.e., most delayed) germination times were noted in the control treatment (T1), with 36.02, 40.51, and 47.02 days.

This significant reduction in germination time under GA_3 and NAA treatments can be attributed to their well-documented physiological effects on seed metabolism and dormancy breaking. Gibberellic acid (GA_3) is known to promote the synthesis of hydrolytic enzymes such as α-amylase, which mobilize stored food reserves in the seed endosperm, enhancing early cell elongation and radicle protrusion (Bewley *et al.,* 2013; Taiz *et al.,* 2015) [1, 16]. The higher concentration (100 ppm) of GA_3 in T4 likely provided sufficient hormonal stimulus to trigger earlier germination compared to lower doses (Rajasekaran *et al.,* 2016; Kumar *et al.,* 2021) [12, 4]. Similarly, Naphthalene Acetic Acid (NAA), a synthetic auxin, supports cell elongation and division and enhances the physiological and metabolic processes required for seedling emergence. At 75 ppm, it possibly optimized these responses without causing inhibitory effects that can occur at higher concentrations (Sarkar and Basu, 2019; Kumar *et al.,* 2021) [13, 4]. Hence, T6 showed slightly delayed but still early germination compared to the control. In contrast, untreated seeds (T1 - Control) took significantly longer to germinate, likely due to the absence of exogenous hormonal stimulation needed to overcome endogenous dormancy and initiate rapid seed metabolism (Sharma and Trivedi, 2014; Taiz *et al.,* 2015) [14, 16].

These results align well with the findings of Sharma and Trivedi (2014) [14], who reported that both GA_3 and NAA accelerated germination in custard apple, with higher doses of GA_3 showing the most pronounced effects. Likewise, Rajasekaran *et al.,* (2016) [12] demonstrated significant reductions in germination time in papaya with GA_3 treatment, and Kumar *et al.,* (2021) [4] further confirmed the positive influence of plant growth regulators like GA_3 and NAA on early and uniform seed germination across various horticultural crops.

Height of seedling (cm) at 60 and 90 DAS

The maximum seedling height at both 60 and 90 days after sowing (DAS) was observed under T4 (Gibberellic Acid @ 100 ppm), with values of 15.25 cm and 30.70 cm, respectively. This was followed by T6 (Naphthalene Acetic Acid @ 75 ppm) at 14.96 cm and 30.46 cm, and T3 (Gibberellic Acid @ 75 ppm) at 14.69 cm and 29.98 cm. In contrast, the lowest seedling height was recorded in the control treatment (T1), with only 11.72 cm at 60 DAS and 22.10 cm at 90 DAS.

This trend indicates the significant role of plant growth regulators, particularly gibberellins and auxins, in promoting shoot elongation and overall vegetative growth. Gibberellic Acid (GA_3) enhances cell elongation by stimulating the expression of genes related to cell wall loosening and expansion, and it promotes internode elongation, resulting in taller seedlings (Taiz *et al.,* 2015; Bewley *et al.,* 2013) [16, 1]. The high efficacy of the

100 ppm concentration (T4) may be due to the optimal hormonal balance it provides for rapid cell division and elongation during early seedling development (Rajasekaran *et al.,* 2016; Kumar *et al.,* 2021) [12, 4]. Naphthalene Acetic Acid (NAA) also contributes to increased seedling height by promoting cell division and elongation in the apical regions of shoots. At 75 ppm, the treatment in T6 appears to have created a favorable auxin concentration that stimulated shoot elongation without triggering any growth inhibition that can occur at higher concentrations (Sarkar and Basu, 2019; Sharma and Trivedi, 2014) [13, 14]. On the other hand, the control treatment (T1), which lacked any exogenous hormone application, resulted in significantly lower seedling height. This suggests that the native levels of endogenous hormones alone were insufficient to stimulate optimal shoot growth under the given conditions.

These findings are in agreement with those of Sharma and Trivedi (2014) [14] in custard apple and Rajasekaran *et al.,* (2016) [12] in papaya, who observed enhanced seedling height under GA_3 and NAA treatments. Kumar *et al.*, (2021) [4] also reported that judicious use of plant growth regulators significantly improved vegetative parameters like seedling height in several horticultural crops.

Diameter of seedling (cm) at 60 and 90 DAS

The maximum seedling diameter at 60 and 90 days after sowing (DAS) was recorded under T4 (Gibberellic Acid @ 100 ppm) with values of 0.356 cm and 0.515 cm, respectively. This was closely followed by T6 (Naphthalene Acetic Acid @ 75 ppm) with 0.335 cm and 0.504 cm, and T3 (Gibberellic Acid @ 75 ppm) with 0.335 cm and 0.494 cm. On the other hand, the minimum seedling diameter was observed in the control treatment (T1), with values of 0.188 cm and 0.326 cm at 60 and 90 DAS, respectively.

The enhanced stem diameter in GA_3 and NAA-treated seedlings can be attributed to the increased mitotic activity, enhanced cell elongation, and secondary tissue development stimulated by these growth regulators. Gibberellic Acid (GA_3) is known to promote stem elongation and girth by stimulating both cell division in the cambium and cell expansion in the cortex and pith (Taiz *et al.,* 2015; Bewley *et al.,* 2013) [16, 1]. The superior performance of GA_3 at 100 ppm (T4) suggests that this concentration provided an optimal hormonal environment for vascular tissue differentiation and radial growth (Rajasekaran *et al.,* 2016; Giri *et al.,* 2021) [12, 2]. Likewise, Naphthalene Acetic Acid (NAA) facilitates cell division and enlargement, particularly in meristematic tissues, thereby contributing to increased seedling thickness. At 75 ppm, it likely maintained a balance between promoting growth and avoiding hormonal stress, leading to significant improvement in stem diameter (Sarkar & Basu, 2019; Jadhav *et al.,* 2017) [13, 3]. The control (T1), lacking exogenous hormonal support, exhibited the least stem thickness, which may be due to limited endogenous hormonal activity under sub-optimal environmental or soil nutrient conditions.

Similar findings were reported by Sharma and Trivedi (2014) [14] in custard apple, and Patel *et al.,* (2020) [9] in guava, where GA_3 and NAA application significantly enhanced seedling girth. Kumar *et al.*, (2021) [4] also confirmed the positive effect of PGRs on increasing the diameter of seedlings in a variety of horticultural crops.

Fresh weight of shoot (g) at 60 and 90 DAS

The maximum fresh weight of the shoot at both 60 and 90 days after sowing (DAS) was observed in T4 (Gibberellic Acid @ 100 ppm), recording 1.73 g and 2.66 g, respectively. This was closely followed by T6 (Naphthalene Acetic Acid @ 75 ppm) with 1.60 g and 2.57 g, and T3 (Gibberellic Acid @ 75 ppm) with 1.56 g and 2.48 g. The minimum shoot fresh weight was noted in the control (T1), at 1.08 g and 1.99 g for 60 and 90 DAS, respectively. This increase in shoot biomass under GA_3 and NAA treatments can be attributed to the growth-promoting effects of plant growth regulators, which enhance both cell division and cell elongation. Gibberellic acid (GA_3) stimulates stem elongation and leaf expansion by promoting hydrolytic enzyme activity and mobilizing stored nutrients, leading to better biomass accumulation in vegetative parts (Taiz *et al*., 2015; Bewley *et al*., 2013) [16, 1]. The superior performance of the 100 ppm concentration (T4) suggests a threshold level at which GA_3 most effectively enhances shoot tissue development without causing hormonal imbalance (Rajasekaran *et al.,* 2016; Giri *et al.,* 2021) [12, 2]. Naphthalene Acetic Acid (NAA) also contributes to improved shoot fresh weight by enhancing photosynthate allocation, promoting apical dominance, and stimulating cellular enlargement in shoot tissues. At 75 ppm, NAA likely created an optimal hormonal environment conducive to robust vegetative growth (Sarkar & Basu, 2019; Jadhav *et al.,* 2017; Patel *et al.,* 2020) [13, 3, 9]. The lowest fresh weight of shoots in the control (T1) could be due to the absence of any exogenous hormonal stimulation, resulting in relatively slower growth and reduced metabolic activity. These results are consistent with earlier findings by Sharma and Trivedi (2014) [14] in custard apple and Kumar *et al.*, (2021) [4] across various fruit crops, where shoot biomass increased significantly following GA_3 and NAA treatments.

Dry weight of shoot (g) at 60 and 90 DAS

The maximum dry weight of shoot at 60 and 90 days after sowing (DAS) was recorded under T4 (Gibberellic Acid @ 100 ppm) with 0.170 g and 0.513 g, respectively. This was statistically at par with T6 (Naphthalene Acetic Acid @ 75 ppm) at 0.157 g and 0.492 g, and T3 (Gibberellic Acid @ 75 ppm) at 0.153 g and 0.460 g. The minimum dry weight of shoot was observed in the control treatment (T1), with 0.106 g and 0.241 g at 60 and 90 DAS, respectively.

The increase in dry matter accumulation under GA_3 and NAA treatments can be attributed to their stimulatory effects on photosynthetic efficiency, nutrient mobilization, and cellular metabolism. Gibberellic acid (GA_3) enhances vegetative growth by increasing the rate of cell division and elongation, leading to higher shoot biomass, which upon drying, reflects as greater dry weight (Taiz *et al.,* 2015; Bewley *et al.,* 2013) [16, 1]. The improved shoot dry weight in T4 suggests that GA_3 at 100 ppm optimally stimulated carbon assimilation and dry matter partitioning toward the aerial parts (Rajasekaran *et al.,* 2016; Giri *et al.,* 2021) [2, 12]. Naphthalene Acetic Acid (NAA), a synthetic auxin, also plays a crucial role in increasing dry matter accumulation by enhancing root-to-shoot hormonal signaling and increasing the efficiency of water and nutrient uptake (Sarkar & Basu, 2019) [13]. At 75 ppm (T6), NAA likely provided the ideal hormonal concentration for active shoot growth and biomass production without inducing physiological stress (Jadhav *et al.,* 2017; Patel *et al.,* 2020) [3, 9]. In contrast, the lower dry weights in the control (T1) reflect sub-optimal growth and metabolic activity due to the absence of exogenous hormonal enhancement. These observations align with previous research by Sharma and Trivedi (2014) [14] in custard apple and Kumar *et al.*, (2021) [4] in multiple horticultural crops, where dry matter production was significantly enhanced by PGRs like GA_3 and NAA.

Table 1: Effect of plant growth regulators and biofertilizers on seed germination and growth parameters of custard apple

Treatment	Days to Germination (Days)	Days to 50% germination (Days)	Days to 100% germination (Days)	Height of seedling at 60 DAS	Height of seedling (cm) 90 DAS	Diameter of seedling (cm) at 60 DAS	Diameter of seedling at 90 DAS	Fresh weight of shoot at 60 DAS	Dry weight of shoot (g) at 60 DAS	Fresh weight of shoot at 90 DAS	Dry weight of shoot (g) at 90 DAS
T_1	36.02	40.51	47.02	11.72	22.10	0.188	0.326	1.08	0.106	1.99	0.241
T_2	31.80	36.46	42.63	13.89	28.14	0.303	0.463	1.48	0.145	2.40	0.408
T_3	29.53	33.97	40.38	14.69	29.98	0.335	0.494	1.56	0.153	2.48	0.460
T_4	28.43	33.19	38.30	15.25	30.70	0.356	0.515	1.73	0.170	2.66	0.513
T_5	32.63	37.32	43.73	13.68	27.44	0.293	0.440	1.45	0.143	2.38	0.387
T_6	28.77	33.31	39.18	14.96	30.46	0.335	0.504	1.60	0.157	2.57	0.492
T_7	31.03	35.71	41.44	14.42	28.95	0.324	0.483	1.50	0.147	2.42	0.450
T_8	34.06	38.55	46.21	13.46	25.59	0.220	0.366	1.38	0.136	2.30	0.345
T_9	33.75	38.20	44.39	13.54	26.80	0.272	0.419	1.40	0.138	2.31	0.356
T_{10}	34.95	39.44	46.88	12.25	24.81	0.199	0.337	1.28	0.125	2.14	0.272
T_{11}	34.29	38.78	46.67	13.02	25.06	0.209	0.356	1.33	0.131	2.23	0.324
S.Em.±	0.062	0.061	0.076	0.026	0.120	0.003	0.003	0.008	0.0004	0.0045	0.0039
CD at 5 % Levels	0.182	0.179	0.225	0.077	0.355	0.008	0.009	0.023	0.0012	0.0134	0.0115

Conclusion

On the basisi of one year research trial it is concluded that the treatment T4 (Gibberellic Acid @ 100 ppm) consistently outperformed all other treatments, producing the earliest seed germination (28.43 days), 50% germination (33.19 days), and full germination (38.30 days), whereas the control (T1) exhibited the most delayed germination (36.02, 40.51, and 47.02 days. Seedling growth parameters were also markedly improved under T4. The maximum height was achieved under this treatment (15.25 cm at 60 DAS and 30.70 cm at 90 DAS), along with the greatest seedling diameter (0.356 cm and 0.515 cm. This treatment further resulted in the highest fresh shoot weights (1.73 g and 2.66 g at 60 and 90 DAS) and dry shoot weights (0.170 g and 0.513 g). Treatments T6 (NAA @ 75 ppm) and T3 (GA_3 @ 75 ppm) followed closely and performed statistically at par with T4 in several parameters, while the control recorded the lowest values across all traits.

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