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Effect of different supplemental pollination methods and pollen storage intervals on fruit yield of custard apple (*Annona Squamosa* L.) cultivar balanagar

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Abstract
Custard apple (*Annona squamosa* L) is, an arid tropical fruit, and its commercial production is limited by the low flower-to-fruit ratio, and asymmetrical shape fruits in consequence of inadequate pollination. A field study was conducted in 2024, during the flowering season at Horticultural Research Station, Adilabad, Telangana with different supplemental pollination methods and pollen grain storage periods on custard apple fruit yields of cultivar Balanagar. Fruit set and fruit quality varied significantly in weight, diameter, and the number of seeds per fruit as a result of pollination methods and pollen storage time. The hand pollination method with the pollen grain stored for 12 hours or 24 hours significantly enhanced the fruit yield and quality characteristics. All the supplemental pollination methods enhanced the fruit yields ranging from 20 to 65%, with symmetrical shapes in Grade 1 to 3 qualities that had high marketability. In conclusion, this research provides further evidence that supplementary pollination is necessary for increasing the yield and fruit quality in commercial custard production.

Keywords: Supplemental pollination, pollen grain storage, fruit set, marketable yields, incremental benefit-cost ratios

1. Introduction
Custard apple or sitaphal (*Annona squamosa* L) referred to as the "fruit of poor people" is one of the most important annonaceous groups of fruits that include bullock heart or ramphal (*Annona reticulata* L), soursop or laxmanphal (*Annona muricata* L) and cherimoya or Hanumanphal (*Annona cherimola* L). Atemoya or pineapple sugar apple is the hybridization of *Annona squamosa* L X *Annona cherimola* L and recently gained importance for its unique flavour, sweetness, and medicinal values. The fruit in anonnaceous family members is an aggregate type developed from a single flower with many ovaries and is often found misshapen, underdeveloped on one side, forming asymmetric fruits, a result of inadequate pollination (Chadha, 2009) [3]. Low marketable yield is a major problem in various anonnaceous fruits, as the fruit size and form depend on the number and position of fertilized ovules. Though custard apple produces a large number of flowers without attractive colour, only a few, 2 to 3% flowers turn into fruits that were linked to hypogyny, protogynous dichogamy, low pollen germination, competition between vegetative and floral growth, extremely high and low humidity during flowering, soil moisture stress, and a lack of insect pollinators (Vinay and Chithirai 2015; Sanghani and Varu, 2022) [27, 24].
Custard apple cultivar Balanagar is a semi-spreading small tree with, basal girths ranging from 20to 45 cm, a semi-spreading crown, and heights between 4.5 and 8.10 meters. The tree was deciduous, leaves oblong or narrow, lanceolate, alternating, and measure 8.26 to 18.50 cm in length and 3 to 6 cm in width. Each flower is 1.5 to 3.50 cm in length, with three petals, and is produced in clusters of three to four. At the bottom of the petal, purple colour spotting can be seen after fully open. In the late afternoon, when the male pollen sacks open, the blossoms are fragrant. Beginning in July, the custard apple begins to bloom, with only a few of the blossoms bearing fruit. It takes 20 to 25 weeks for the fruit to mature in hot, semi-arid conditions.

Beginning in October and continuing through November, custard apples are harvested, and typically two or three pickings of the fruits (Yadav, 2018) [28].

Custard apple natural stands are diminishing faster in various parts of Telangana primarily due to the conversion of land into real estate ventures and, degradation of forests. Previously regarded as a wasteland crop, it is now grown as an orchard on saline, heavy, rocky, gravel, and sandy soils (Meena *et al*., 2022) [16]. The price of the fruits is also remunerative to the farmers as the fruits are primarily consumed as fresh and pulp was used in puddings and ice creams. In India, custard apple was cultivated in 51,720 hectares, with 4.6 lakh tonnes of fruits produced per year. In Telangana, custard apple was commercially grown on 930 hectares with a fruit yield of 9480 tonnes during 2022-2023 year. New orchards are established every year and to increase the fruit set and marketable yields, evolving supplemental pollination methods is essential to maximize the returns. Hand pollination improves fruit set and substantially boosts fruit yield per tree by increasing the fruit size and number. However, it is labour intensive and should be adopted in varieties that produce premium quality fruits by choosing efficient pollen source. Pollen viability is crucial for fruit set and efficient pollination (Marlon *et al*., 2014) [14]. The amount of pollen that reaches the stigma is important; the highest pollen germinability on the stigma was seen during open pollination, indicating that overloading the stigmas during supplemental pollination may increase competition among pollen grains and decrease yield (Vaknin *et al*., 2002) [26]. Ultimately fruit yield and quality were greatly boosted above natural levels by supplemental pollination methods (Pritchard and Edwards, 2006) [19].

The separation of the external petals apex marks the beginning of anthesis, which takes six to eight hours to complete anthesis. Stigma is receptive from the day before anthesis until two to three hours after anthesis, but then it decreases rapidly. By the time of dehiscence, stigma is nearly unreceptive, and protogynous dichogamy was observed. Pollination methods and pollen storage conditions resulted in a significant variation in fruit shape and size and marketable quality. Hand pollination increased fruit set percentages by more than 80% when female flowers were picked late in the afternoon and hand pollination was carried out eighteen hours later (Marlon *et al*., 2014) [14]. With economically marketable yields and acceptable fruit quality in commercial plantations, appropriate supplemental pollination techniques are required (George, 1998) [9].

One of the custard apple commercial cultivar Balanagar selection was developed by Fruit Research Station, Sangareddy, Telangana, and is the premium variety recommended for commercial cultivation in Telangana. The fruits of this cultivar are asymmetrical, often pyramid-shaped, average number of fruits of 51.4 / tree, fruit weight of 137 to 264 g, and seed weight of 5.7 g/fruit. The good quality fruits with high pulp content of 44.9%, Total Soluble Solids of 20.7 ºBrix, acidity of 0.20%, reducing sugar of 15.0%, and total sugars of 17.9% (Radha and Mathew, 2006) [20]. The effective period for hand pollination is limited, and the success of hand pollination is dependent on environmental factors such as wind and rainfall. Furthermore, hand pollination is a labour-intensive process resulting in high labour costs. As an alternative technique, spray pollination using aqueous pollen grain solutions is expected to reduce labour and costs in fruit tree cultivation.

The source of carbohydrates needed for pollen tube expansion and pollen germination is sucrose. The culture medium's sugar has the role of keeping the medium's osmotic pressure stable, and it acts as a substrate for pollen metabolism (Patel and Mankad, 2015) [18]. When pollination is a limiting factor in pistachio orchards, supplementary pollination using pollen suspension can be used commercially (Karimi *et al*., 2017) [13]. Therefore, several attempts have been made to establish spray pollination methods to reduce the time and labour costs of pollination. The spray pollination method takes less than half of the time needed for hand pollination and the amount of the pollen grain required for the spray pollination was about one-third of that needed for the hand pollination. The objectives of the research were to evolve appropriate supplemental pollination methods to enhance fruit set percent, marketable shape and size, and quality of the custard apple cultivar Balanagar.

2. Materials and Methods

2.1 Fruit orchard selection

The pollination study was conducted in the custard apple fruit orchard established in 2016 at Horticultural Research Station, Adilabad, Telangana during the 2024 flowering season. Trees were planted at 4 m x 4 m in the square system. Eight-year-old, well-grown, uniform stature trees of custard apple cultivar Balanagar were selected for the pollination study.

2.2 Pollen collection, storage and pollination methods

The pollen was collected from the partially opened flowers and refrigerated at 4 ºC. To prepare 10% sucrose solution, 10 g sucrose was dissolved in 100 ml of de-ionized water. We used 500 mg pollen per liter suspended in 10% sucrose solution or de-ionized water as it was established for Pistachio crop in increasing fruit size (Vaknin *et al*., 2002) [26]. Camel hair brush was used to smear pollen grains on stigmas in the hand pollination treatments, and a pressurized hand-held sprayer was used in treatments with pollen suspension sprays. Pollen suspension was sprayed twice for every partially opened flower. The practice of different supplemental pollination as per treatments was conducted between 8:00 AM to9.00 AM at early female phase.

2.2 Supplemental pollinations methods

Pollination methods (M): Four methods tested were M_0: Natural pollination, M_1 Hand pollination with camel hair brush, M_2: Pollen suspension in deionised water using hand held sprayer, M_3: Pollen suspended in 10% sucrose solution using hand held sprayer.

Pollen storage intervals (S): Three intervals studied were S_o: Natural, S_1: 12 hours and S_2: 24 hours

2.3 Estimation of fruit yield and Quality parameters

Number of fruits per tree was calculated by counting all fruits at the time of harvest. The weight of five fruits was measured with the help of electronic balance 0.01 g accuracy. Fruit length and fruit diameters were measured with the help of verniercaliper and expressed in milli metres. Pulp, seeds and rind were separated and weights were recorded. The number of seeds per fruit was calculated by counting the seeds of five fruits and the average was recorded as number of seeds per fruit. Stone fruits were calculated on per plant basis. Total stone-converted fruits were calculated and expressed in percentages. Total Soluble Solids (TSS) was determined by using a hand refractometer and expressed in ºBrix. The fruit shapes normal and misshapen were visually graded from 1 to 5 scale proposed by George and Nissen (1988) [9]. The commercial and marketable fruits fit in the grades 1, 2 and 3, while 4 and 5 were non-commercial and unmarketable fruits. The shape grades 1: highly symmetrical, 2 symmetrical, 3 partially symmetrical, 4 irregular and 5

completely irregular.

$$\text{Pulp percentage (\%)} = \frac{\text{Pulp weight}}{\text{Total fruit weight}} \text{ X } 100$$

$$\text{Pulp to Seed ratio} = \frac{\text{Pulp weight}}{\text{Seed weight}} \text{ X } 100$$

$$\text{Incremental Benefit Cost Ratio} = \frac{\text{AdditionalIncome}}{\text{AdditionalCostofCultivation}}.$$

2.4 Statistical Analysis

A randomized block design, with four replications (each replicate contains a tree) factorial concept (FRBD) was used. For analysis of fruit characteristics, each replication contains five fruits means. Analysis of variance (ANOVA) was performed using the PAST 4.03 statistical software (Hammer and Harper DA, 2001) and if the effects of the treatments were significant (p<0.05), then the means were compared by Tukey's HSD test.

3. Results and Discussions

The F-statistic in ANOVA table and P values indicated supplemental pollination methods, pollen grain storage intervals had significant effects on fruit set%, fruit yield and quality characteristics studied (Table 1).

Significant differences were observed in fruit set percentages. The highest fruit set percentage was recorded in hand pollination with camel hair brush with pollen grain stored for 12 hours and 24 hours. Pollen suspension with 10% sucrose and de-ionised water are stastically similar with each other to natural pollination. The variation in different treatments might be due to proper fertilization and pollen tube germination with camel hair brush or pollen suspension sprays compared to natural pollination. These results are similar to the other researchers Hayes *et al*. (2017) [11], Sanghani and Varu (2022) [24] and Meena *et al*. (2023) [15] reported on custard apple and other fruit crops.

Hand pollination and pollen suspension with 10% sucrose and de-ionsed water with pollen grain storage for 12 hours and 24 hours had significant differences observed in physical parameters i.e., fruit length, fruit diameter, fruit weight, pulp weight, seed weight, percent stone fruits. This might be due to better distribution of pollen on stigmas of all of the female flowers more than to an additional contribution of pollen. Such type of variability was recorded by Ullah *et al*. (2018) [25] in date palm, Nor *et al*. (2019) [17] in apple, and Meena *et al*. (2023) [15].

As fruit length and width were more in fruits it might be resulted in increased fruit weight. Also might be due to artificial pollination which improves cell division and cell enlargement, leading to an increase in fruit size and weights (Mohammadi *et. al*. 2017) [13]. These results are in conformity with the researchers Saleh *et al*. (2014) [22], Ullah *et al*. (2018) [25] and El-Sharabasy *et al*. (2020) [7] in date palm.

The greater pulp weight might be due to pollen sources affecting the growth of ovarian tissues concerning hormones released by growing endosperm and embryo tissues, which diffuse into the ovarian tissue and exert a specific effect on the fruit growth. These results are in corroborating with Saleh *et al*. (2014) [22], Ullah *et al*. (2018) [25] and Meena *et al*. (2023) [15]. Rind weight might be due to larger fruit size and volume due to proper pollination and fertilization might affect the weight of the rind. The results are in accordance with the finding of Meena *et al.*, (2023) [15] observations of higher rind weight for hand pollination in custard apple Arka Sahan with pollen of Balanagar cultivar.

Pollen sources and pollen viability from the related species can affect fruit set, seed weight, and quality of fruits (Chander and Kurian, 2019) [5]. These results are in conformity with the similar variations observed by Jalikop and Kumar (2007) [12] and Atawia *et al*. (2016) [2] in citrus and Meena *et al*. (2023) [15]. The number of pollen grains and the development of ovules might be a possible reason for variation in seed number per fruit among all the treatments (Chander and Kurian, 2019) [5]. Percentage of stone fruits were reduced in different supplemental pollination methods and pollen grain storage of 12 hours and 24 hours compared to natural pollination (Control).The similar results were reported by Sanghani and Varu DK (2022) [24]. Insignificant differences were recorded in pulp percentage, pulp: seed ratio. Total soluble solids were more in supplemental pollination methods with pollen grain storage of 12 and 24 hours compared to natural pollination. Hand pollination and pollen source significantly affected sweetness of fruits as reported by Jalikoop and Kumar (2007) [12] in custard apples. These results were also similar to other researchers Samnegard *et al*. (2019) [23] and Meena *et al*. (2023) [15].

Significant differences were observed in number of fruits per tree and yield per tree. More number of fruits per tree and highest yield were recorded in hand pollination and pollen suspension with 10% sucrose and deionsed water with pollen grain storage for 12 hours and 24 hours compared to natural pollination. The results of the higher number of fruits per tree might be due to the higher percentage of fruit set as a result of better fertilization with hand pollination with camel hair brush at different pollen storage times. Similar results of variability in the treatments were also found by Saleh *et al*. (2014) [22] and Sanghani and Varu (2022) [24]. The treatments with supplemental pollination showed significantly higher fruit yields in comparison with natural pollination. These results were in conformity with Elrefaey and Eldengawy (2014) [6] and Ullah *et al*. (2018) [25] in date palm and Sanghani and Varu (2022) [24] in custard apple.

Table 1: Analysis of variance (ANOVA) of P-values for supplemental pollinations methods and pollen storage periodson fruit yield and quality of custard apple cultivar Balanagar

Factors	Fruit set%	Fruit length (mm)	Fruit diameter (mm)	Fruit weight (g)	Pulp weight (g)	Pulp (%)	Rind weight (g)	Seed weight (g)	Stone Fruits%	Number of seeds per fruit	Pulp: seed ratio	Total Soluble Solids (^{0}Brix)	Number of fruits per tree	Fruit yield per tree (kg)
Pollination method (M)	0.001	<0.001	0.001	<0.001	0.003	<0.001	0.033	0.008	<0.001	0.030	0.040	<0.040	<0.001	0.001
Storage interval (S)	0.033	0.008	<0.001	0.030	0.040	<0.040	<0.001	0.004	0.003	<0.001	<0.001	<0.001	0.001	0.004
MXS	<0.001	0.004	0.003	<0.001	<0.001	<0.001	0.001	<0.001	0.001	<0.001	0.003	<0.001	0.033	<0.001

Table 2: Effect of supplemental pollination methods and pollen storage periods on fruit yield and quality of custard apple cultivar Balanagar

Treatment	Fruit Set%	Fruit Length (Mm)	Fruit Diameter (Mm)	Fruit Weight (G)	Pulp Weight (G)	Pulp (%)	Rind Weight (G)	Seed Weight (G)	% Stone Fruits	Number of seeds per fruit	Pulp: Seed Ratio	Total Soluble Solids (^{0}Brix)	Number of fruits per tree	Fruit yield per tree (Kg)
M_1S_1	72.90a	69.45a	73.90a	168.35a	82.60a	48.95	66.00a	19.75a	2.32e	38.25	4.44	23.35	168.45a	25.50a
M_1S_2	69.00a	66.00ab	66.58b	142.45b	69.95b	49.03	57.50ab	15.00abc	3.23de	48.30	5.10	23.11	156.35ab	21.23b
M_2S_1	57.90b	62.31bc	64.18b	121.00c	52.77c	43.50	51.73bc	16.50abc	5.90bc	45.45	3.52	22.16	147.00b	14.84d
M_2S_2	55.40 b	58.27cd	61.50bc	106.75d	46.50cd	43.61	46.50cd	13.75abc	6.92b	44.55	3.47	21.89	132.75c	12.21e
M_3S_1	60.50b	64.70abc	64.13b	128.75bc	56.50c	43.62	54.00bc	18.25ab	4.49cd	44.25	3.71	22.39	154.75ab	18.10c
M_3S_2	59.70b	61.11abc	56.85cd	103.25d	47.25cd	45.58	43.75cd	12.25bc	5.75bc	36.25	4.23	21.87	129.25c	14.60d
M_0S_0	60.00b	53.87d	51.20d	87.00e	38.75d	44.35	38.00d	10.25c	11.03a	43.75	3.78	20.16	113.00d	9.37f
SE.m ±	2.69	1.55	1.39	3.01	2.56	1.82	2.22	1.58	0.37	3.48	0.57	0.13	3.01	0.38
CD at 5%	8.01	4.59	4.15	8.95	7.61	NS	6.596	4.68	1.09	NS	NS	0.40	8.95	1.13
CV%	8.67	4.97	4.46	4.92	9.09	8.01	8.69	20.85	12.93	16.21	28.04	1.22	4.21	4.59

Values followed by different letters in a column are significantly different by Turkey's HSD test at $p \leq 0.05$

Pollination methods: M_0: Natural pollination, M_1 Hand pollination with camel hair brush, M_2: Pollen suspension in deionised water using hand held sprayer, M_3: Pollen suspended in 10% sucrose solution using hand held sprayer.

Pollen storage intervals (S): S_o: Natural, S_1: 12 hours and S_2: 24 hours

Table 3: Effect different supplemental pollination methods on incremental yield, additional cost of cultivation, additional income and incremental benefit cost ratio over natural pollination control

Treatments	Incremental Yield (Kg/Ha)	Additional cost of cultivation (Rs/Ha)	Additional Income (Rs/ Ha.)	Incremental Benefit Cost Ratio
M_1S_1	8081	8000	2,04,062	25.50
M_1S_2	7412	8000	1,70,625	21.32
M_2S_1	3418	4000	1,70,937	42.73
M_2S_2	1775	4000	1,22,750	30.68
M_3S_1	6456.	4055	2,72,812	67.27
M_3S_2	6268	4055	1,63,437	40.30

Pollination methods: M0: Natural pollination, M1 Hand pollination with camel hair brush, M2: Pollen suspension in deionised water using hand held sprayer, M3: Pollen suspended in 10% sucrose solution using hand held sprayer.

Pollen storage intervals (S): So: Natural, S1: 12 hours and S2: 24 hours

4. Conclusion

The custard apple flowers have numerous pistils and fertilization with viable pollen is a prerequisite to increase fruit set, fruit size, and fruit quality. Under the Northern Telangana Agroclimatic Zone, pollination during May to June months was found to be effective. Fruit set and quality of custard apple cultivar Balanagar were significantly affected by the pollination method and pollen grain storage intervals. Hand pollination significantly enhanced the fruit set and marketable yields and not practicable on large scale. As an alternative, 500 mg/ litre pollen suspension in 10% sucrose solution may be advocated custard fruit set and marketable quality fruits.

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