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Evaluation of hand pollination and natural pollination on fruit set of acid lime (*Citrus aurantifolia* Swingle.)

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Abstract
Among the various citrus species, one of the commercially important species is acid lime (*Citrus aurantifolia* Swingle). Poor pollination and fertilization are the major reasons for initial fruit drop. Climate change and intensive agriculture, including pesticide use during flowering, can hinder insect-mediated pollination and ultimately affects the fruit set. Hence, understanding of pollination ecology and the efficacy of various pollination strategies under novel conditions is critical for enhancing fruit yield under precision farming. However, instead of directly evaluating pollination efficiency, many researchers use alternative methods such as manual pollination and pollen sprayers. This study investigated the impact of hand pollination on fruit set and retention in acid lime compared to open pollination. The results showed significant differences in fruit parameters, including fruit set, retention, diameter, length, circumference, and weight. Open pollination yielded the highest fruit set (66%), followed by hand pollination (64%) and natural covered pollination (46%). However, hand pollination with self-pollen resulted in the highest fruit retention at maturity (63.32%), surpassing open pollination (46.07%) and natural self-pollinated flowers (23.66%). Fruit parameters like diagonal fruit circumference (10.56, 7.96, and 11.1 cm), longitudinal fruit circumference (11.08, 8.32, and 20.64 cm), fruit diameter (3.1, 2.76, and 3.3 cm), fruit length (3.54, 3.04, and 3.7 cm), and fruit weight (21.6, 12.2, and 23 g/fruit) were highest in hand pollination followed by open pollination and lowest in natural covered pollinated flowers. These findings suggest that hand pollination can improve fruit yield, particularly in situations where open pollination is hindered by unpredictable weather conditions during flowering.

Keywords: Hand pollination, open pollination, fruit set, fruit retention, yield

Introduction

Among the various citrus species grown in the tropics, one of the most important and commercially grown citrus species is acid lime (*Citrus aurantifolia* Swingle) after the mandarins and sweet oranges. It is said to have originated in the Hindustan center, possibly in North East India and Myanmar and is now grown in mild subtropical and tropical regions spanning from the northern plains to the central highlands and southern coastal areas, all with hot semi-arid climates and black and red soils. It can be grown effectively from sea level to 1500 meters above mean sea level. The tree is susceptible to cold, and its ideal temperature range is 20 to 30 °C.

The acid lime is an evergreen and ever bearing tree. The juvenile phase lasts about 2-3 years. The flowers, either hermaphrodite or staminate, are produced on new flushes at the axils of the leaves. As the flower opens, the stigma becomes receptive and remains so for a few days. The pollen is not released until the flower opens. Insect-mediated self-and cross-pollination occurs. However, fruit set is limited due to self-incompatibility in some varieties. Citrus species bloom prolifically, producing as many as 100,000-200,000 flowers on a mature tree. Fewer than 1-2% of these flowers produce a harvestable fruit. (Erickson and Brannaman, 1960) [8]. Initial fruit set and subsequent fruit drop are the major factors which affect the ultimate yield of lime. The initial fruit drop is due to weak flowers and fruit lets with defective styles and ovaries, improper pollination and inability to produce parthenocarpy fruits. Later, it is due to environmental factors and endogenous hormones. Pollen has a clear effect on fruit setting, final fruit retention, seediness, and fruit growth, as well as various physiological activities during fruit set and development (Dhillon *et al*., 1961; Soost, 1956; Mustard *et al*., 1956) [7, 20, 15].

In recent years, there has been debate about the relationship between various specialized and extensive plant pollinators and their floral characteristics prediction abilities as the most effective pollinator indicator of plant species. Insects are the major pollinators in many plants including citrus. However, globally, habitat loss and fragmentation due to urbanization has had numerous negative impacts on pollinator diversity. Furthermore, the increased application of pesticides, herbicides, and chemical fertilizers, as well as the effects of diseases and parasites (Basu *et al*., 2014; Desneux *et al*., 2007; Evison *et al*., 2012; Vanbergen *et al*., 2018) [4, 6, 9, 23], has had negative repercussions on pollinators, as have decreasing plant diversity and climate change (Vanbergen *et al*., 2013, Cameron and Sadd, 2020) [22, 5]. Hence, understanding of pollination ecology and the efficacy of various pollination strategies under novel conditions is critical for enhancing fruit yield under precision farming. However, instead of directly evaluating pollination efficiency, many researchers use alternative methods such as manual pollination and pollen sprayers. Thus, the present study was undertaken to understand the effect of hand pollination on fruit set and retention of acid lime.

Material and Methods

The present experiment was conducted at the Instructional Farm, Faculty of Agriculture, Bidhan Chandra Krishi Vishwavidyalaya, Mohanpur, West Bengal, during 2020-2021. The experiment was conducted during the *Ambe bahar* flowering season, using ten flowers per treatment on a single tree. The treatments were as follows: T_1-Emasculated flowers bagged without pollination; T_2-Open pollination; T_3-Natural covered pollination (natural pollination with its own pollen in a covered environment using a butter paper bag); and T_4-Hand pollination with own pollen grains. For T_4, ten flower buds were selected and enclosed in a paper bag before anthesis, in the evening from 4:30 pm to 5:30 pm, after removing the anthers from the bud. The following morning, when the stigmas were yellow and sticky sugary secretions oozed from the stigmatic surface, the paper bags were removed, and pollen from the same flower was gently dusted onto the stigmas using a paint brush. To prevent undesired pollination by insects or wind, these pollinated flowers were labeled and re-bagged using paper bags (Figure 1). Observations were recorded for fruit set, fruit retention, fruit drop, fruit weight, diameter, and other relevant characteristics

Pollen viability assessment

Pollen viability was assessed using the acetocarmine staining method. Freshly opened male and hermaphrodite flowers were collected and placed in Petri dishes to induce pollen dehiscence by exposure to sunlight for 10-15 minutes. Subsequently, the dehisced pollen grains were transferred to glass slides by gentle tapping and stained with 1-2 drops of 1% acetocarmine. After a 10-minute incubation period, the slides were examined microscopically. Viable pollen grains, characterized as normal, well-filled, and fully stained, were counted alongside non-viable pollen grains, identified as unstained or poorly developed. Counts were performed across ten randomly selected microscopic fields. The percentages of viable and non-viable pollen grains were then calculated based on the total pollen grain count.

$$\text{Pollen viability (\%)} = \frac{\text{No of stained pollengrains}}{\text{Total no of pollengrains}} \times 100$$

Pollen germination study

For *in vitro* pollen germination assays, freshly dehisced pollen grains were cultured on a germination medium composed of 30% sucrose and 100 ppm boric acid. The sitting drop technique was employed using cavity slides, with three replicate drops per slide. Following incubation for 6-8 hours in Petri dishes containing moistened germination paper to maintain humidity, pollen germination was assessed using a light microscope. The percentage of germination was determined by examining ten randomly selected microscopic fields per slide. Subsequently, slides were stained with Alexander's stain for preservation and future analyses.

$$\text{Pollen germination (\%)} = \frac{\text{No of pollen grains germinated}}{\text{Total no of pollengrains}} \times 100$$

Data analysis

The data was analyzed using MS Excel and software OPISTAT. The level of significance $p<0.05$ was considered.

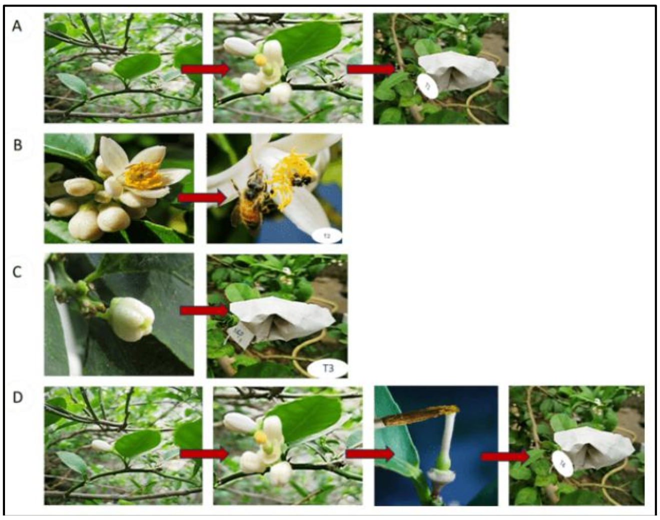

Fig 1: Procedure followed for all the treatments. A-Emasculated flowers bagged without pollination (T_1), B-Open pollination (T_2), C-Natural covered pollination (T_3), D-Hand pollination with own pollen grains (T_4)

Results and Discussion

Pollen viability: Hermaphrodite flowers showed 72.54% viability and 65.8% germination, whereas staminate flowers showed 69.39% viability and 60.5% germination, indicating that hermaphrodite flowers produce more fertile pollen compared to male flowers.

Table 1: Pollen viability and Pollen germination percentage in hermaphrodite and male flower

Sl. No	Flower type	Viability (%)	Germination (%)
1	Hermaphrodite flower	72.54	65.8
2	Staminate flower	69.34	60.5

Stigma receptivity

Stigma receptivity was assessed by visual inspection of the stigmatic surface for the presence of a sticky substance. In acid lime, the stigma was observed to be receptive well before anthesis (90%) and remained receptive for up to 24 hours. These findings are consistent with the report by Mishra and Dash (2019) [13]. High stigma receptivity was noted on the day of anthesis until 1:00 pm, after which receptivity began to decline. A drastic reduction in receptivity was observed one day after anthesis (Mishra and Dash 2019) [13].

Fruit set

The experimental findings indicated that the highest incidence of fruit set was observed in treatment group T2, with an average of 6.6 fruits per 10 flowers. This was followed by treatment group T4, which exhibited an average of 6.4 fruits per 10 flowers. In contrast, treatment group T3 demonstrated a lower average fruit set of 4.6 fruits per 10 flowers, while treatment group T1 showed a complete absence of fruit set. These values correspond to percentage fruit sets of 66%, 64%, 46%, and 0% for T2, T4, T3, and T1, respectively. However, statistical analysis revealed no significant difference in fruit set between treatment groups T2 and T4 (Table 2, Fig 2, Fig 3). Similar results were also obtained by Motial (1964) [14] in acid lime, Similarly, Shrivatsava and Pathak (1993) [17] in Aonla. However, contrast result was obtained by Bapaji (1968) [2] in Aonla, King *et al*. (2007) [11] in Asparagus, Samnegard *et al*. (2019) [16] in apple, showed hand pollination was superior. Atawia *et al*. (2016) [1] reported that emasculation, and bagging treatment showed lowest fruit set in orange and Li and Zhang (2007) [12] in Aonla. Apart from these, Hasegawa and Nakajima (1990) [10] reported that hand pollination had no effect on increasing the fruit set percentage in persimmon. In the present investigation, hand pollination showed less fruit set than open pollination, possibly because of damage to the floral parts during emasculation or insufficient pollen grains for fruit set.

Fruit retention

Regarding fruit retention, all treatments showed significant differences. The highest percentage after three months of pollination was observed in the hand pollination treatment (63.32%), followed by open pollination (46.07%) and natural covered pollination (23.66%). Conversely, the lowest percentage of fruit drop was recorded in the hand pollination treatment (50%), compared to open pollination (53.93%) and natural covered pollination (76.33%) Table 2, Fig 2, Fig 3). Present data on fruit retention is supported by studies of Atawia *et al*. (2016) [1], in which hand pollination with March grapefruit pollen grains and Balady mandarin pollen grains produced higher fruit retention compare to open pollination. In contrast Singh *et al*. (1998, 2001) [18, 19] reported that highest fruit retention in open pollination followed by sibbing (selfing with one genotype) and geitonogamy (selfing within one plant).

Table 2: Effect of pollination modes on fruit set, retention and drop

Sl. No.	Treatments	Fruit set	Percentage of fruit set	No of fruit retained after 3 months of pollination	Percentage of fruit retained	No of fruit dropped	Percentage of fruit dropped
1.	T_1	0.000	0.000	0.000	0.000	0.000	0.000
2.	T_2	6.600	66.000	3.000	46.070	3.600	53.930
3.	T_3	4.600	46.000	1.200	23.666	3.400	76.334
4.	T_4	6.400	64.000	4.00	63.316	2.400	50.004
SE m(±)		0.346	3.464	0.292	5.590	0.381	6.509
CD (5%)		1.047	10.475	0.882	16.903	1.151	19.681

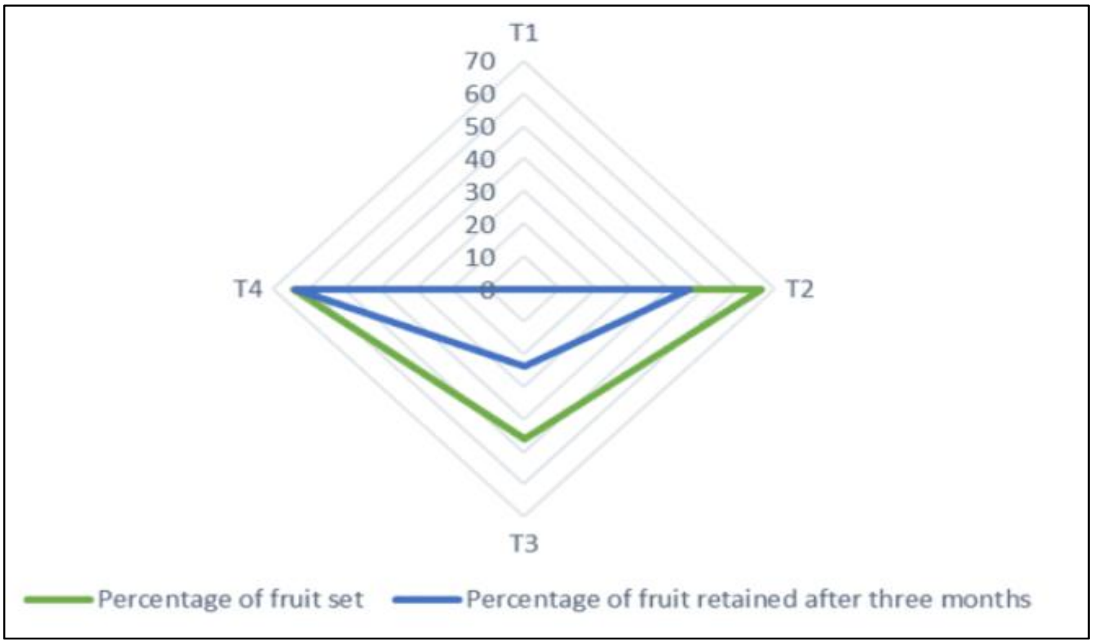

Fig 2: Percentage of fruit set after 7 days of pollination and percentage of fruit retained after 3 months of pollination.

Fig 3: Fruit set after seven days of pollination (a, b, c, d) and Fruit retained after three months of pollination (e, f, g).

Fruit weight, diameter, length and circumference

Perusal of the data from the present experiment showed that the hand pollination treatment resulted in the maximum fruit weight (23 g), fruit diameter (3.34 cm), and fruit length (3.78 cm), followed by open pollination, which showed 21.6 g fruit weight, 3.1 cm fruit diameter, and 3.54 cm fruit length. Natural covered pollination showed the least fruit weight (12.2 g), fruit diameter (2.7 cm) and (3.04 cm) fruit length. However, there was no significant difference between hand pollination and open pollination with respect to fruit weight and fruit diameter. Both longitudinal and diagonal fruit circumference showed significant differences among the treatments. These measurements were highest in hand pollination (20.64 cm and 11.1 cm), followed by open pollination (11.08 and 10.56 cm) and natural covered self-pollination (8.32 and 7.96 cm), indicating that pollen grains have a significant effect on fruit marketable quality and that hand pollination was superior (Table 3 and Figure 4). Similar results were also obtained in yellow passion fruit (Wilfredo *et al*., 2020) and Kiwi fruit (Vaissiere, 1991) [21].

Table 3: Effect of different pollination modes on fruit diameter, length, fruit circumference

Sl. No.	Treatments	Fruit weight (g)	Fruit diameter (cm)	Fruit length (cm)	Longitudinal fruit circumference	Diagonal fruit circumference (cm)
1.	T_1	0.000	0.000	0.000	0.000	0.000
2.	T_2	21.600	3.160	3.540	11.080	10.56
3.	T_3	12.200	2.760	3.04	8.320	7.960
4.	T_4	23.000	3.340	3.780	20.64	11.100
SE (m)		0.806	0.074	0.073	0.391	0.147
CD (5%)		2.438	0.213	0.222	1.182	0.444

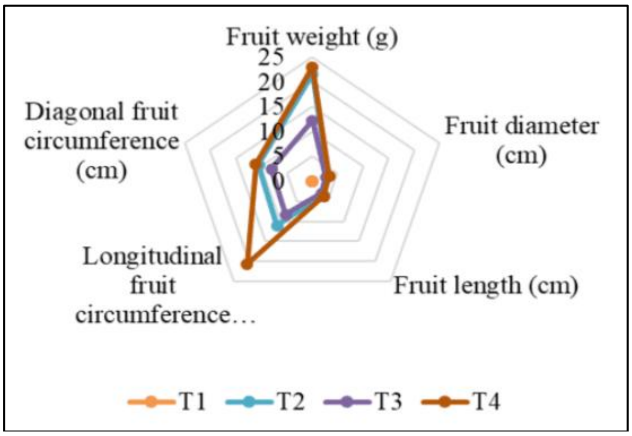

Fig 4: Influence of pollination methods on fruit weight, diameter, length, fruit circumference

Conclusion

Thus, the present investigation concluded that both open pollination and hand pollination are effective in achieving higher yields of acid lime. While open pollination exhibited the highest initial fruit set, hand pollination with self-pollen demonstrably enhanced fruit retention to maturity and positively affected various fruit quality attributes in acid lime. These findings suggest that hand pollination represents a potentially beneficial technique for optimizing fruit yield and quality in acid lime, particularly in unfavorable climatic conditions that hamper insect pollinators for effective pollination, when considering the cost-benefit ratio of hand pollination. In future, it is necessary to study the effect of hand pollination on the number of days required for fruit maturity, fruit yield, and the physicochemical properties of fruits, and to standardize the appropriate timing and frequency of hand pollination to obtain maximum benefits. The cost-benefit ratio of the treatments should be considered before recommendation. There is scope to study artificial pollination by pollen sprayers, ladders and hydraulic lift bloom dusters by considering the cost benefit ratio.

Declaration of Competing Interests

The authors declare no competing financial interests.

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