Research Article

Pollination Improve Physico-Chemical Properties of Litchi Fruit (*Litchi chinensis*)

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Abstract | Litchi holds significant importance as a global fruit crop due to its rich content of essential vitamins such as C, B-complex, and flavonoids, which play a crucial role in maintaining a balanced human diet. The process of pollination, a vital ecosystem service, substantially contributes to enhancing the nutritional quality and yield of cross-pollinated fruits and vegetables. To evaluate the impact of bees and syrphid flies on enhancing the physical and biochemical characteristics of litchi fruit, an experimental investigation was conducted in a Litchi orchard situated in Multan, Pakistan. The study encompassed an examination of pollinator diversity and abundance, as well as their foraging behavior, including the time spent and rate of visits. Additionally, diverse pollination treatments were compared, pitting instances of unguided insect visits against scenarios where insects were excluded. This comparison encompassed both physical attributes (such as fruit dimensions, weight, pulp content, and seed count per fruit) and biochemical parameters (including Total Soluble Solids (TSS), Titrable Acidity (TA), pH, and Vitamin C). The pollinator community observed consisted of two species of bees and eight species of flies. Among these, *Episyrphis balteatus* emerged as the most prevalent insect pollinator, closely followed by *Apis florea* and *Calliphora sp*. The highest visitation rate was recorded for *Apis dorsata*, followed by *E. balteatus* and *Ischiodon scutellaris*. Comparative analysis revealed that fruit dimensions, weight, pulp content, and seed count per fruit were notably improved in instances where insect pollination was facilitated, in contrast to cases where no insect visits were allowed. Furthermore, chemical properties were also found to be superior in scenarios involving free insect visits. The study underscored the efficacy of *A. dorsata* and syrphid flies (*E. balteatus* and *I. scutellaris*) in terms of visitation rates. Thus, conserving these insect pollinators bears the potential to significantly augment litchi fruit yield in the Punjab region of Pakistan.

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1. Introduction

It is a highly valued fruit that is consumed fresh or processed into jams, jellies, and juices. Litchi is an important fruit that is cultivated worldwide for its nutritional value and economic significance. Its increasing popularity in the global market highlights its importance, and the high yields it produces make it an attractive crop for farmers. Litchi, known for its rich content of vitamin C, fiber, and antioxidants, offers a range of health benefits (Singh *et al*., 2020). According to FAO, global litchi production surged from 2.2 million tons in 2017 to 2.4 million tons in 2020, marking a 9% growth over three years (FAO, 2021). China, India, and Vietnam lead in litchi production, with China contributing to over 70% of the total yield (Wang *et al*., 2021; Singh, *et al*., 2020).

The formation of litchi inflorescence occurs on terminal branches, resulting in panicles carrying an abundance of yellowish-white flowers, arranged in a sequence of male and female blossoms. Three distinct types of litchi flowers exist, differing in development and function (Mustard *et al*., 1953; Stern and Gazit, 1998). The timing of litchi flower anthesis varies due to weather conditions. While hermaphrodite flowers are typically self-sterile, they necessitate insect pollination for fruitful results, drawing various insect visitors like honeybees, ants, wasps, and flies due to the copious nectar present (Pandey and Yadava, 1970; Phadke and Naim, 1974; Groft, 1943).

In the context of cross-pollination, insects play a pivotal role in pollen transfer, consistently regarded as the most effective method for achieving optimal litchi production (DuToit, 1994; Stern and Gazit, 1996; Menzel and Waite, 2005). *Apis* and *Melipona* spp. Consist of 98-99% of total visitors however, *Apis mellifera* emerging as a crucial and primary litchi pollinator (Pandey and Yadava, 1970; McGregor, 1976; Vithanage and Ironside, 1986; Vithanage, 1986).

The European honey bee *A. mellifera* has been identified as a notably effective insect pollinator, contributing to the substantial enhancement of litchi crop production (Kumar and Kumar, 2014). In an effort to boost fruit set, four honey bee colonies were introduced into a litchi orchard during the flowering period, resulting in a two to three-fold increase in fruit set within open inflorescences compared to those enclosed in cages (Badiyala and Garg, 1990). Similarly, a separate investigation indicated that the most prolific fruit setting occurred in open treatment, followed by cross treatment, while self-pollination exhibited significantly lower fruit set in litchi fruit (Kumari *et al*., 2018). Within litchi orchards, four distinct species of syrphid flies have been documented as the dominant pollinators, displaying more robust foraging behavior compared to honey bee species such as *A. cerana, A. dorsata* and *A. mellifera* (Rai *et al*., 2017). Prior research has underscored the prevalence of syrphid flies as primary pollinators over a two-year study conducted in a litchi orchard (Dubby *et al*., 2020).

Compare with self-pollination, cross pollination have positive impact on physio-biochemical properties of many fruit crops i.e., date palm (Shafique *et al*., 2011), in strawberry fruits (Klatt *et al*., 2013) and tomato (Bashir *et al*., 2018). Strawberry flowers are visited by many insect pollinators strawberry flower have less specialized characters i.e., radial symmetry, anthers are exposed and nectar is easily accessible (Nye and Anderson, 1974; Albano *et al*., 2009). Insect pollination is very important for strawberry as it increases the yield and improves fruit quality (Garibaldi *et al*., 2014). It has been observed in strawberry that insect pollination enhances the physiological properties of strawberries like increases the fruit weight, gives more intense color to the fruit, less malformations and a longer shelf life than not pollinated with insects (Klatt *et al*., 2014; Wietzke *et al*., 2018). Open pollination (free insect visit) enhances the physico-biochemical properties such as number of fruit, fruit weight, fruit retention, fruit size, pulp/seed ratio, pulp weight, juice %, vitamin C and T.S.S. (total soluble salt) % in shaddock fruit as compared to self-pollination and emasculation and caged treatments (Jannila *et al*., 2020).

The significance of insect pollination in enhancing both the yield and quality of litchi fruit has been well established. Past research has predominantly focused on the influence of managed honey bees on litchi. Consequently, the present study was devised to assess the contributions of alternative insect pollinators, namely wild honey bees and syrphid flies, in litchi fruit production. This investigation aimed to uncover their effects on not only the quantity but also the physical and biochemical attributes of litchi fruit, thereby expanding our understanding beyond the scope of previous studies.

2. Materials and Methods

2.1 Study area

The research was conducted between March and May of 2019 at the Khakwani Horticulture orchard on Dunyapur Road in the Multan district of Punjab, Pakistan (29.79N, 71.71E). The experimental focus was on litchi cultivation, and the designated experimental area covered one acre. The litchi orchard was surrounded by indigenous trees like Acacia sp. and Amultas. Multan, situated in a subtropical zone, experiences high temperatures during the summer (ranging from 35 to 40°C) and chilly winters (8 to 10°C). The annual rainfall averages between 127-254 mm, and the winter season is characterized by dense fog (Khan and Hasan, 2019).

2.2 Pollinators abundance

Pollinator presence and variety were documented on a daily basis across five distinct time slots (0800, 1000, 1200, 1400, and 1600 hrs). Concurrently, measurements of relative humidity and temperature were taken. From a pool of litchi trees, ten (10) were randomly selected to gather data on pollinator abundance. A timed observation approach, employing a stopwatch, was utilized to record all insect visitors present around each litchi tree for a duration of one minute. The data collection commenced when approximately 10% of the litchi flowers had bloomed in the field. Throughout the litchi tree's flowering season (March-May, 2019), insect pollinators were captured using hand nets and subsequently classified to the lowest feasible taxonomic level. This classification was facilitated by referring to identification keys for bees and syrphid flies (Vockeroth, 1969; Michener, 2000).

2.3 Foraging behavior

Insect foraging behavior of abundant pollinators of litchi were assessed in term of number of flowers visited by an insect in one minute (visitation rate) and time spent by an insect pollinator on one flower (visit duration) by using a stopwatch along with recording of relative humidity and temperature during each census (Sajjad *et al.*, 2019).

2.4 Comparison of different pollination treatments

There were two pollination treatments i.e., self-pollination (no insect visit) and cross pollination (free insect visit). For this purpose, thirty (30) inflorescences were caged with muslin cloth bag from each direction (East, West, North and South) of litchi tree before anthesis for self-pollination. For cross-pollination data, randomly thirty (30) inflorescence were selected from different directions (East, West, North and South) of litchi tree(s). To compare self and cross pollination, fruit were harvested from different treatments and brought to the laboratory for analysis of different yield parameter of litchi fruit.

2.5 Physical parameters of litchi fruit

First of all, total number of fruits per panicle were counted in both self and cross pollination. At the maturity stage, randomly 20 fruits were selected at same maturity level for measuring physical parameters of litchi fruits (fruit length, fruit weight, pulp weight and seed weight/fruit) in each replication from the cross and self-pollination treatment.

2.6 Biochemical parameters of litchi fruits

Four different biochemical parameters were taken which include Total soluble solid (TSS), Titrable acidity (TA), pH and vitamin C. TSS were measured by using refactrometer from the juice extracted from the mature harvested fruit. Data were averaged and recorded as percent TSS. Moreover, Titrable acidity (TA) were measured in laboratory, 10 ml juice of fruit were taken in a beaker from each treatment and diluted (1:4) with distilled water. Titratable acidity was determined by titrating against 0.1N NAOH after adding 2-3 drops of phenolphthalein as an indicator (Akhtar *et al.*, 2010). pH reading were taken by using Digital pH-meter for calculate the acidity of fruit juice. Furthermore, vitamin C (Ascorbic acid) of litchi juice was calculated by method of titration against (2,6-dichlorophenol indophenol) following the method of AOAC (Anonymous, 1990).

2.7 Analysis

In the context of a Randomized Complete Block Design (RCBD) experimental layout, the data pertaining to visit duration and visitation rate were subjected to Analysis of Variance (ANOVA) for analysis. Subsequent comparisons of means were conducted through the Least Significant Difference (LSD) test at a significance level of P=0.05. Furthermore, a comparison between open pollination and pollinator exclusion treatments concerning both the physical attributes (fruit length, fruit weight, pulp weight, and seed weight per fruit) and the biochemical properties (Total Soluble Solids (TSS), Titrable Acidity (TA), pH, and vitamin C) of litchi was executed using a paired sample t-test at a significance

Table 1: Insect species visiting Litchi along with their abundance, proportion and visitation frequency in flowers.

Order	Family	Genus/species	Total abundance	Proportion	Visitation frequency (Individuals/ inflorescence/minute)
Hymenoptera	Apidae	*Apis florea*	2944	31.8	9.81
		Apis dorsata	661	7.1	2.20
Diptera	Syrphidae	*Episyrphis balteatus*	3197	34.5	10.66
		Ischiodon scutellaris	805	8.70	2.63
		Eristalinus aeneus	309	3.34	1.03
		Eristalinus laetus	92	0.99	0.31
		Syrphus ribesii	64	0.69	0.21
		Eupeode corollae	29	0.31	0.09
		Scava latimacullata	43	0.46	0.14
	Calliphoridae	*Calliphora* sp.	1103	11.92	3.67

level of P=0.05. All statistical analyses were executed using Minitab 16 software (Ryan *et al*., 2012).

3. Results and Discussion

3.1 Pollinators abundance

In present study, pollinator community was comprised of eight syrphid fly species (*Episyrphis balteatus*, *Ischiodon scutellaris, Eristalinus aeneus, E. balteatus, Syrphus ribesii, Eupeode corolla, Scava latimacullata* and *Calliphora* sp.) belonging to two families and two honey bee species (*Apis florea* and *A. dorsata*) species. Overall, Diptera and Hymenoptera comprised 61% and 39% of total pollinator's abundance in litchi orchard, respectively. Among all pollinators *E. balteatus* (Syrphidae: Diptera) was most abundant followed by *A. florea* (Apidae: Hymenoptera). While *E. corolla* and *S. latimacullata* were found least abundant. Similarly, visitation frequency of *E. balteatus* was highest followed by *A. florea* (Table 1).

3.2 Foraging behavior

Foraging behavior were assessed in term of visitation rate and visit duration. In present study, the highest visitation rate was recorded for honey bees followed by syrphid flies. The visitation rate (no. of floret/ inflorescence/sec) of *A. florea* (9.00) was significantly higher followed by *I. scutellaris* (7.50) and *Calliphora* sp. (6.00). While *A. dorsata* (2.80) has least visitation rate. Moreover, remaining pollinators have non-significant difference in visitation rate. Furthermore, *E. aeneus* (5.48) has significantly higher visit duration followed by *A. floria* (3.82) while *A. dorsata* (2.12) has least visit duration (Table 2).

3.3 Physical properties of litchi

T-test showed significant differences in physical properties of litchi fruit between cross pollination and self-pollination. In cross pollination there was significant increase in average fruit set/branch ($P < 0.001$), fruit length (cm) ($P < 0.001$), fruit diameter (cm) ($P < 0.001$), pulp weight (g) ($P < 0.001$) and seed weight (g) ($P < 0.001$) and other parameter i.e., total fruit weight (g) and time to reach market maturity than self-pollinated fruits. We observed more than 53% fruit set in cross pollination as compared to self-pollination (Table 3).

Table 2: Pollination effectiveness of six pollinators in terms of visitation rates and stay time on Litchi flowers.

Insect pollinator	Visit duration (Time spent per floret)	Visitation rate (No. of inflorescence visited/minute)
Apis florea	3.82 ± 0.35 b	1.00 ± 0.25d
Apis dorsata	2.12 ± 0.21c	5.50 ± 0.85a
Episyrphis balteatus	3.22 ± 0.29 bc	3.00 ± 0.52b
Ischiodon scutellaris	3.07 ± 0.23bc	2.80 ± 0.51bc
Eristalinus aeneus	5.48 ± 0.85a	2.50 ± 0.64 bcd
Calliphora sp.	3.29 ± 0.36 bc	1.20 ± 0.52cd

Means followed by the same letters in a column are not statistically different according to LSD test at 5% level (± S.E.).

3.4 Chemical properties of litchi

Paired sample T-test revealed significant differences in chemical parameters i.e. TA ($p = 0.01$), TSS ($p = 0.000$), pH ($p= 0.01$) and Vitamin C ($p = 0.03$) of litchi fruit between cross pollination and self pollination. In cross pollination total soluble solid TSS (26.61 ppm) is greater than self pollination treatment (17.68 ppm). While in self pollination the value of titrable acidity (1.43 g/L) and vitamin C (340.74 mg) is higher than cross pollinate on titrable acidity (0.92 g/L) and vitamin C (191.53 mg) (Table 4).

Table 3: Comparison of physical characteristics of self and open pollinated fruits in litchi.

Pollination	Avg. fruit set/ branch	Time to reach market maturity (days)	Fruit length (cm)	Pole diameter (cm)	Equatorial Diameter (cm)	Total fruit wt. (g)	Pulp Wt. (g)	Seed Wt. (g)
Cross pollination	7.17±1.42	63.91±3.84	31.09±0.54	26.49±0.33	24.79±0.27	21.24±0.54	13.74±0.35	3.58±0.16
Self-pollination	4.67±1.13	67.53±0.84	28.86±0.41	24.85±0.28	22.41±0.27	17.54±0.38	11.38±0.30	2.85±0.13
t (Observed value)	2.43	1.72	1.68	2.43	2.51	2.43	3.91	2.58
|t| (Critical value)	1.98	1.98	1.98	1.98	1.98	1.98	1.98	1.98
DF	19	19	19	19	19	19	19	19
p-value (Two-tailed)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Means (± S.E.).

Table 4: Comparison of chemical characteristics of self and open pollinated fruits in litchi.

T-test results	TSS (ppm)		TA (g/L)		pH		Vitamin C (mg)	
	Cross	Self	Cross	Self	Cross	Self	Cross	Self
Means ± S.E.	26.61±2.19	17.68±0.12	0.92±0.22	1.43±0.04	3.68±0.00	3.63±0.01	191.53±9.55	340.74±9.02
t (Observed value)	11.24		4.07		7.42		11.36	
|t| (Critical value)	1.98		1.98		1.98		1.98	
DF	19		19		19		19	
p-value (Two-tailed)	0. 01		0.0035		0.01		0.03	

Means (± S.E.).

Most dominant and abundant pollinator order in present study was Diptera followed by Hymenoptera. Similar results were recorded in litchi orchard where species of Diptera was most abundant (Bhatia *et al.*, 1995). In another study, syrphid flies were also recorded efficient and dominant pollinator in litchi crop (Dubby *et al.*, 2020). In contrast, the prevalent pollinator in the realm of Litchi was *A. dorsata* (50.11%), with *A. cerana* following suit (Das *et al.*, 2019; Abrol, 2006), and *A. mellifera* (26.84%) coming next (Rai *et al.*, 2017). Conversely, in other fruit crops like avocado (Perez-Balam et al., 2012) and strawberry (Castle *et al.*, 2019), syrphid flies took precedence, trailed by honey bees in abundance. Within the current study's context, the longest visit duration was exhibited by *E. aeneus*, closely followed by *A. florea*. This finding stands apart from earlier research, which indicated *A. dorsata* as the most prolonged visitor to litchi flowers (Das *et al.*, 2019; Matangi and Neeraj, 2018). Furthermore, the highest visitation rate in terms of inflorescences visited per minute was attributed to *A. dorsata*, whereas the highest florets visited per minute was observed with *A. florea*, followed by syrphid flies. This outcome is corroborated by Das *et al.* (2019), where *A. florea* displayed the highest visitation rate (12.29) among all pollinators.

Bees and flies collectively contributed to elevating the quality and yield of litchi crops (Kumar and Kumar, 2014). In the present study, the physical attributes of fruit production, namely fruit length, fruit weight, pulp weight, and seed weight per fruit, were notably higher in cross-pollinated inflorescences. Both bee-pollinated and open-pollinated litchi crops exhibited a substantial enhancement in fruit set and fruit quality (Matangi and Neeraj, 2018). In contrast, cage plants without pollinators demonstrated significantly reduced fruit set per panicle across various crops (Cayuela *et al.*, 2011). Similarly, in other fruits, cross-pollination emerged as a catalyst for superior quality, larger size, and heightened fruit setting in strawberry (Abrol *et al.*, 2019), coconut (Melendez-Ramirez *et al.*, 2004), guava (Rajagopal and Eswarappa, 2005), as well as pomegranate fruit (Derin and Eti, 2001; Tao *et al.*, 2010).

Honey bee is an effective floral visitor of litchi cultivar and ensures high litchi production (Chaturvedi, 1965; Das and Choudhury, 1958). Two to three times higher fruit setting was observed where four colonies of honey bees were introduced in litchi orchard at the time of inflorescence compared to caged inflorescence (Badiyala and Garg, 1990). Pollination through augmentation of *Apis mellifera* honey bee increased the fruit weight 113% and 56% in natural pollination (Khan *et al.*, 2012).

Cross pollination enhanced the physico-chemical properties (number of fruits, fruit weight, fruit retention, fruit size, pulp/seed ratio, pulp weight, juice %, vitamin C and T.S.S.% in the fruits as compared to other pollination treatments (self-pollination, emasculation and caged) (Jannila *et al*., 2020). Our result showed that insect pollinators positively affected physico-chemical properties of litchi. Bee pollination increased sugar acid and vitamin C content in Litchi previously (Liang *et al*., 2018). It increase the TSS values, aril weight (an extra seed covering) and seed pulp ratio of litchi fruit (Matangi and Neeraj, 2018). Open pollinated strawberry plants gave the fruit of more red in color, more in weight, better in size and with superior physio-chemical properties as compared to those without any pollinators. Better TSS and TA values were also observed in open pollinated strawberry (Abrol *et al*., 2019). Present results are similar with previous studies where honey bee pollination played a significant role in improvement of physico-chemical properties of apple varieties i.e., Anna and Dorsett Golden (Yehia *et al*., 2008; Shaheen *et al*., 2017) and increased the number of fruits, fruit weight and TSS percentage in *Aegle marmelos* commonly called bael fruit (Satapathy and Chandra, 2018; Karimi and Mirdehghan, 2015).

Conclusions and Recommendations

Since *Apis dorsata* and syrphid flies (*Episyrphis balteatus* and *Ischiodon scutellaris*) proved the most effective in terms of visitation rate, therefore, conserving these insect pollinators can enhance litchi fruit yield in Punjab, Pakistan.

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Novelty Statement

No previous study has been reported from South Punjab Pakistan to explore effectiveness of native insect pollinators in improving the quality of this tropical fruit.

Authors Contribution

MA, AS and MAA: Conceived and designed the experiments. QA, MAA and MA: Performed the experiments. MAA and MA: Analyzed the data. QA, MA, AS and SS: Contributed reagents, materials, analysis tools. QA, MAA and MA: Wrote the paper. All the authors commented on previous versions of the manuscript.

Conflict of interest

There are no conflicting interests in relation to the publication of this article.

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