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Pollination efficacy of honeybee (*Apis mellifera*) influencing litchi fruit production

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Abstract
Present studies were conducted at local litchi orchards in Muzaffarpur, Bihar, from flowering (March) to harvesting (May) of litchi, during 2023. To understand the impact of wild pollinators and *Apis mellifera* on pollination and fruit setting of litchi, different experimental pollination treatment were used to quantify qualitative and quantitative characters of litchi. Decline in density of female flower to mature fruit with some variation in all treatments except nylon-bagged inflorescence that produces only 0-2 fruits with minimal qualitative characteristics, per inflorescence were recorded. Fruit setting along with other qualitative character such as fruit weight, peel weight, pulp weight, pH and TSS were higher (20.55 g, 2.19 g, 15.28 g, 4.76 and 22.43 ºBrix respectively) in open pollination system with *A. mellifera* under litchi tree. Based on recorded data productivity can be sequence as pollination treatment with *A. mellifera* hive under the tree > closed pollination treatment with *A. mellifera* hive inside nylon cage > open pollination in *A. mellifera* hive were 500 meter apart > open natural pollination without *A. mellifera* hive and at last bagged inflorescence. Resultant data show gradual decrease in fruit setting as the hive move away from experimental inflorescence. The result clearly indicate the pollination efficiency of *A. mellifera*.

Keywords: Pollination, *A. mellifera*, fruit quality, fruit set, litchi

Introduction
Litchi (Sapindaceae) is a Southeast Asian tropical fruit. It has traditionally been farmed as a high valuable fruit crop. Lychee output has lately increased in tropical and subtropical locations around the world. China is the world's largest producer of litchi, followed by India, Vietnam, and Thailand. Litchi is grown on an area of 92,100 hectares in Assam, Bihar, Jharkhand, Odisha, Uttarakhand and West Bengal and with an annual output of 583,400 t. Bihar is India's largest litchi producing state, accounting for approximately 37% of both national area and production. Muzaffarpur, Bihar, is known as 'Litchi City' because of its shahi litchi.

Litchi is cross-pollinated and highly entomophilic tree requires insect for pollination and fruit development because of self-sterility (Pandey & Yadav 1970, Phadke & Naim 1974) [16, 17]. Litchi inflorescence consists of interspersed. Three types (pure male, hermaphrodite female and hermaphrodite male) of flowers (Stern & Gazit 1996) [20]. Each types of flower opens at different time; firstly; pure male, secondly; hermaphrodite female and at last hermaphrodite male (Stern & Gazit 1996) [20]. Male flowers opened in daylight between 8 and 16 hours (Malhotra *et al*., 2018) [15].

Nectar rich flora are the peculiar characteristic of self-sterile flower, which attract diverse insect species. Depending on availability of different floral sources nearby, foraging behaviour of different pollinator vary significantly (Ahmad *et al.*, 2021; Bashir *et al.*, 2018 [1, 2]. Honeybee pollination of entomophilous crops has been considered as one of the most effective and cost-effective methods for increasing agricultural yield and quality (King *et al*., 1989) [12]. Honeybees have been recognised as the most beneficial insect visitor on litchi (Groff, 1943) [10]. Honeybees have been identified as a key pollinator of litchi by certain studies, owing to their frequent and effective flower visits (Davenport and Stern 2005) [20]. Domestic hives of Asian honeybee *A. cerana* and European honeybee *Apis mellifera* have been used in some industrial litchi plantations in India and China to promote pollination and output (Davenport & Stern 2005 [7]; Kumar & Kumar 2014 [13]. (i) Present study was carried out to investigate Quantification of pollination success and fruits development (ii) Effect of different pollination treatments on qualitative characters (iii) Litchi yield in different pollination treatment.

Materials and Methods

Experimental region

Observations were done at individually managed litchi orchards at two locations 26°09'09"N 85°14'06"E • 79 m and 26°05'46"N 85°26'17"E • 78 m, Muzaffarpur, Bihar, India during litchi blooming (March first- second week) to litchi harvesting period (third – fourth week) 2023.

Quantification of pollination success and fruits development

Number of female flower was counted at four stages to estimate the success of pollination and ultimately development of fruit. Stage 1: Newly bloom female flower were counted just before applying any treatment. Stage 2: Recent developing ovary, a very young fruits were counted after second week. Stage 3: Developing young fruits were counted at third week. Stage 4: Finally, matured fruit, about to harvest were counted after 8th week.

Impact of different treatment on yield of litchi

To evaluate the impact of *A. mellifera* on success of pollination, fruit setting and ultimately on yielding of litchi fruits, five treatment were employed in four replication. In this experiment, 20 trees were assigned and were arranged in Randomised Block Design. Among 5 treatments, two were closed and three were open system.

To exclude all wild pollinators a control system was design. It was a cylindrical cage of nylon having fine mesh was installed with the support of light metal wire. Such structure act as barrier between flower and pollinator. One inflorescence bagged in one cage (T_1). A litchi tree with a domesticated *A. mellifera* beehive was covered with a fine nylon mesh cage of 15*15*15 feet height (T_2). Open natural pollination system without any domesticated *A. mellifera* beehive (T_3). Open pollination system with a domesticated *A. mellifera* bee hive under the litchi tree (T_4). Tree account for study located 500 meter apart from installed *A. mellifera* hive (T_5).

Each tree form all treatment, four inflorescence of approximate uniform size in four directions (North, East, South and West) were labelled with ribbon. All treatments were taken out when all flowers were faded the new very young fruits were commenced. Treatments were employed for approximate 20-22 days.

Quantification of qualitative and quantitative parameter of litchi

After harvesting, measurement of different of different qualitative and quantitative parameter of litchi were taken account. Weight of whole litchi fruit, seed peel and pulp were weighted using electronic balance. Length of fruit and seed were measured by using Vernier calliper. TSS and pH of pulp was measured by using brix refractometer and pH meter respectively.

Statistical analysis

The statistical analysis was done in factorial RBD. Statistical analysis was done with the help of Microsoft Excel 2016 and OPSTAT. To test the significance of mean difference Analysis of Variance (ANOVA) at 5% used.

Results and Discussion

Quantification of pollination success and fruits development

Data were recorded from blooming of flower to yield of litchi, accounting various intermediate stages. The data listed in table 1 showing the pollination success rate of different treatments in year 2023. Pollination success show significant variation in different treatments. Total 80 inflorescence were tagged in 5 treatments (each treatment have 16 experimental inflorescence). Across all 80 measured inflorescence an average 165 female flower opening synchronously on about mean 0.55 m rachis length.

Table 1: Estimation of pollination success, development of young to mature fruit under the influence of different experimental treatment during 2023

Treatment	Mean no. female flower/penicle	Mean no. fertilized ovule/penicle (%)	Mean no. young fruit/penicle (%)	Mean no. mature fruit/penicle (%)
T_1	165	-	-	1.43 (0.9)
T_2		37.95 (23)	33 (20)	24.75 (15)
T_3		23.1 (14)	16.5 (10)	11.55 (7)
T_4		42.9 (26)	34.65 (21)	28.08 (17)
T_5		29.7 (18)	21.45 (13)	14.85 (9)

The number of fertilised ovules and young fruits were not recorded in the closed (T_1) system. 0-2 fruits were recorded per inflorescence in this treatment. Nylon mesh acts as an insect barrier, and these 0-2 fruits may be produced as a result of pollen transport via wind or mechanical processes. In the T_1 system, an average of 1.43 (0.9%) ripe fruits were produced.

Similarly, in a closed system with *A. mellifera* (T_2) pollination treatment, from 188 flowers 37.95 (23%) of the female flowers successfully fertilised, 33 (20%) grew into immature fruits, and 24.75 (15%) developed into mature fruits. In the open pollination (T_3) condition, 23.1 (14%), 16.5 (10%), and 11.55 (7%) female flowers changed into fertilised ovules, early fruit, and finally mature fruit, respectively. Furthermore, in open pollination with *A. mellifera* (T_4), the number of fertilised ovules, immature fruit, and mature fruit formed from 188 (mean) flowers was 42.9 (26%), 34.65 (21%), and 28.05 (17%), respectively.

Finally, only 14.85 (9%) of 188(mean) flowers grew into mature fruits in the T_5 treatment, whereas 29.7 (18%) and 21.45 (13%) of total female flowers transformed into fertilised ovules and young fruits, respectively.

On the basis of above result we can sequenced the treatments as $T_4 > T_2 > T_5 > T_3 > T_1$. T_1 treatment clearly proof the self-sterility of litchi because despite of full blooms, the bagged inflorescence unable to produce fruit setting. This result proves the finding of Pandey and Yadava (1970) [16], Phadke and Naim (1974) [17] that litchi flower are self-sterile and entomophilous. In one tree different inflorescence bloom at different day but bloosming phase within inflorescence always-same (Stern & Gazit; 1996) [20], whereas Chaturvedi (1965) [20] reported that the number of flower and blooming

were dependent on age of tree and environmental condition. The resultant data clearly show that the fruit setting increases when *A. mellifera* hive was placed in litchi tree. Mature fruit setting gradually decreases as we move apart from the *A. mellifera* hives. This result was may be due to foraging of *A. mellifera* more frequent in T_4 treatment where hive is under the tree. More foraging by *A. mellifera* provide better pollination service, which ultimately leads to better fruit setting. Maximum fruit setting among all treatments was observed in T_4 treatment where box was under the litchi tree. Kumar and Kumar (2014) [13] reported similar result.

Quantification of qualitative and quantitative parameter of litchi

To estimate the role of honeybee *A. mellifera* on quantitative and qualitative character different pollination treatment were employed during litchi blooming period of 2023. The effect of different pollination treatments on quantitative and qualitative characters has been depicted in table 2.

Table 2: Effect of different pollination treatments on qualitative characters of litchi during 2023

Treatments	Mean of fruit size			Mean of seed size			Peel weight (g)	Pulp weight (g)	Total soluble solid (Brix)	pH of juice
	Weight (g)	Length (cm)	Breadth (cm)	Weight (g)	Length (cm)	Breadth (cm)				
T_1	12.18_e	2.37_c	2.12_d	1.74_d	1.64_d	1.06_d	1.03_e	9.26_e	18.51_d	2.76_d
T_2	19.69_b	3.48_b	3.15_b	3.67_a	2.31_a	1.51_a	1.94_c	13.96_b	22.26_b	3.64_c
T_3	19.18_c	3.44_b	3.14_b	2.97_b	2.19_b	1.29_b	2.11_b	13.63_c	20.75_c	4.56_b
T_4	20.55_a	3.67_a	3.17_a	2.81_c	2.21_b	1.19_c	2.19_a	15.28_a	22.43_a	4.76_a
T_5	18.25_d	3.44_b	3.12_a	2.79_c	2.13_c	1.13_c	1.84_d	13.38_d	20.86_c	4.51_b
C.D. (p=0.05)	0.46	0.20	0.03	0.28	0.10	0.08	0.39	0.45	0.17	0.22
SE(m)	0.15	0.06	0.01	0.09	0.03	0.02	0.13	0.14	0.05	0.07
SE(d)	0.21	0.09	0.02	0.13	0.05	0.03	0.18	0.20	0.08	0.10
C.V.	163	3.90	0.73	6.34	3.05	4.03	13.83	2.21	0.52	3.52

Fruit size

Fruit produced in T_4 treatment was larger (weight; 20.55 gm, length; 3.67 cm and breadth; 3.17 cm) in all other treatment. Fruit size (weight; 19.69 gm, length; 3.48 cm and breadth; 3.15 cm) produced by T_2 treatment was significantly less than T_4 treatment whereas, significantly larger than T_3 treatment (weight; 19.18 gm, length; 3.44 cm and breadth; 3.14 cm). T_5 treatment produces fruit size (weight; 18.25 gm, length; 3.44 cm and breadth; 3.12 cm) lesser than fruit produced by natural pollination system. Finally the fruit size (weight; 12.18 gm, length; 2.37 cm and breadth; 2.12 cm) produced by controlled system was significantly smaller among all treatments. Fruit length of T_3 and T_5 treatments were significantly similar whereas fruit breadth of T_2 and T_3 were significantly similar.

Seed size

Seed size (weight; 3.67 gm, length; 2.31 cm and breadth; 1.51 cm) of litchi produced by T_2 was significantly greater followed by T_3 treatment (weight; 2.97 gm, length; 2.19 cm and breadth; 1.29 cm). T_4 treatment produces seed size of weight; 2.81 gm, length; 2.21 cm and breadth; 1.19 cm. Seed size (weight; 2.79 gm, length; 2.13 cm and breadth; 1.11 cm) of litchi produces by T_5 treatment was significantly, lesser size of seed developed in controlled pollination system. Seed weight of T_4 and T_5 were significantly similar. Similarly, seed length of T_3 and T_4 were significantly similar whereas seed breadth of T_4 and T_5 were significantly similar.

Peel weight

Measurement of litchi peel weight (2.19 gm) produced in open pollination system with a *A. mellifera* bee hive under the litchi tree was significantly greater than all other treatment. Lesser peel weight was observed in inflorescence bagged in nylon mesh.

Pulp weight

Pulp the actual edible part of litchi fruit show maximum weight (15.18 g) in T_4 treatment followed by pulp weight (13.96 g) of litchi T_2 treatment. Pulp weight were 13.83 g and 13.38 g in T_3 and T_5 treatment respectively. Lesser pulp weight (9.26 g) recorded in bagged inflorescence

Total Soluble Solid (TSS) & pH

The TSS and pH (22.43 ºBrix and 4.76 respectively) were significantly higher in T_4 treatment among all treatment. The TSS and pH (18.51 ºBrix and 2.76 respectively) were significantly lesser than all other pollination treatment.

Litchi yield in different pollination treatment

The percentage of fruit setting and average fruit weight among all pollination treatment were significantly higher in open pollination system with a *A. mellifera* bee hive under the litchi tree which ultimately result in crop yield. Yield in case of inflorescence bagged in nylon mesh was almost negligible because only 0-2 fruit setting/ inflorescence and their weight was about half weight of fruit produced by other treatments.

Yield of litchi not only depend on pollination success but also on agro-climatic condition during flowering and fruit development period. Present studies clearly indicate that managing of *A. mellifera* can increases yield qualitatively as well as quantitatively. Yield in open pollination system with a *A. mellifera* bee hive under the litchi tree and litchi tree caged in fine nylon mesh with a *A. mellifera* box were almost similar. Similarly yield in open natural pollination system without any *A. mellifera* beehive and tree located 500 meter apart from installed *A. mellifera* hive. Various workers performed similar works. Maximum fruit size (weight, length and breadth) were recorded in natural pollination and pollination in tree caged with *A. mellifera* reported by Rai and Shrivastava (2012) [18].

In the present study value of TSS ranges 21-22.68 which is slightly higher than recording of previous worker. Srivastav *et al*., (2017) [19] recorded 18.32-19.33 ºBrix from different treatments whereas; Kumar and Kumar (2014) [13] recorded 20.1-20.8 ºBrix. Dinesh *et al*., (2014) found that TSS content increases with increase in storage period of litchi. Kumari *et al*. (2023) [14] reported 21.86-22.3 ºBrix TSS in different treatments.

A. mellifera is significant pollinator of litchi flowers. No or negligible fruit set were observed in inflorescence bagged

inside nylon mesh. Present study proves the finding of previous workers (Chaturvedi; 1965, Butcher; 1957, Das and Chaudhary; 1958) [20, 4, 6] no fruit set was produced by inflorescence covered with nylon bag. Havier fruits produced from cross pollination where as in self-pollination fruits produced were lighter in weight (Stern *et al*., 1996) [20]. Badiyala and Awasthi (1991) [3] reported that pollination of litchi by honeybee yield maximum (38 kg/tree) as compared to yield of litchi without bee pollination (9.6kg/ tree). All results clearly show the importance of *A. mellifera* in increasing litchi yield.

Conclusion

We can conclude from result, yield of litchi not only depend on pollination success but also on agro-climatic condition during flowering and fruit development period. Present studies clearly indicate that managing of *A. mellifera* can increases yield qualitatively as well as quantitatively. Yield in open pollination system with a *A. mellifera* bee hive under the litchi tree and litchi tree caged in fine nylon mesh with a *A. mellifera* box were almost similar.

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