

Diversity of Pollinator Species and Effect of Pollination on Strawberry Yield and Quality

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ABSTRACT

Purpose: Insects constitute an important pollinator resource, and they are a key driver for both the fruit yield and the quality. It contributes in ensuring complete pollination and reproductive success of strawberries. However, the comprehensive benefits of insect pollinators in strawberry yield and fruit qualities through pollination are not quantified through empirical evidences. Therefore, this study quantifies the influence of pollinators on strawberry production.

Research Method: The experiment was set up with two treatments (i.e., T_1 - Open plots of strawberry, T_2 – Mesh screen caged plot) arranged in a randomized complete block design with four replications per treatment. Opportunistic visual encounter and observational plot methods were followed to collect the insect pollinators sample. Further, the complete enumeration sampling was applied to collect strawberry plants sample.

Findings: The results showed that during strawberry blossom, Tetramorium sp. was the prominent visitor (n = 506) followed by Apis cerana (n = 322). Based on the insects' foraging behavior, insect abundance and visit frequency, Apis cerana, Apis laboriosa, Xylocopa acutipennis, Bombus sp. and Junonia almana were recorded to be an effective Camarosa pollinator. The exclusion of pollinators in Camarosa resulted in smaller-sized and deformed fruits with inferior fruit quality attributes (color, shape and total soluble solids) compared to open plot fruits. The fruit quality attributes were significantly affected by pollination.

Originality/ Value: This study is the first of its kind to record the insect pollinators visiting Camarosa cultivar and study reported Tetramorium sp. as a successful pollinator in strawberries for the first time.

Keywords: Camarosa, Pollinator diversity, Strawberry pollination

INTRODUCTION

Strawberry (*Fragaria ananassa* Duch.) is a widely grown fruit species of the genus *Fragaria*, belonging to the Rosaceae family. It is one of the most commercially produced fruit and widely preferred for its nutritional value, sweet aroma and flavor (Oguz *et al.*, 2022). Globally, the strawberry production is reported to increase from 6.5 million metric tonnes to 8.9 million metric tonnes between 2009-2019 (FAO, 2021). According to the FAO (2020), annual worldwide production of strawberry was 8.8 million metric tonnes and based on the continent, Asia is the major strawberry producer with 4.1 million

metric tonnes.

In Bhutan, strawberries were introduced with the release of Chandler cultivar in 2006 and Sweet Charlie and Camarosa in 2014 (Ngawang, 2017). Due to increasing demand for fresh fruits and jam,

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cultivation of strawberries at commercial scale is gaining momentum in the country. However, mass production of strawberries is constrained by several challenges, including factors such as poor fruit quality, pests and diseases, climate change, unreliability of pollinating insects and inadequate pollination (Abrol *et al.*, 2017).

Strawberries are a pollination-dependent crop and they must undergo pollination (Abrol *et al.*, 2017). Therefore, insect pollinators are considered to be one of the most effective and inexpensive methods to improve strawberry yield and quality (Bagnara and Vincent, 2015). They are essential to ecosystem and agricultural fields, and are associated with enhanced fruit quality and yields in agricultural settings (Hopwood *et al.*, 2015). Studies have shown that an estimated 85% of the world's flowering plants and 35% of global crop production rely on animal and insects for pollination (Ollerton *et al.*, 2011; Maus *et al.*, 2017).

Insect pollinators play a crucial role in strawberry pollination and appear to be economically important (Ahrenfeldt *et al.*, 2015). However, there is growing concern about the declining diversity of pollinators and its potential impact on strawberry production worldwide, due to factors such as habitat loss, pesticide exposure, and climate change. Understanding the diversity of pollinator species and their role in strawberry pollination is important to ensure optimal fruit yield and quality. Therefore, there is a need to investigate and understand the diversity of pollinator species visiting strawberry flowers and their effect on strawberry yield and quality.

Although the flowers of modern cultivated strawberries are predominantly hermaphrodite flowers capable of self-fertilization (Albano *et al.*, 2009), cross-pollination still occur as these flowers may not be completely self-fertile (McGregor, 1976). Moreover, the stigmas of strawberry flower usually become receptive before the anthers of the same flower release pollen, which necessitates cross pollination. Therefore, pollen-carrying vectors such as living insects are crucial for cross pollination to occur

(Klatt *et al.*, 2014a). Cross-pollination can result in an increase of 62% in the number of fertilized achenes and for proper fruit set in strawberries, it requires at least 70–80% of pistil (female part) to be pollinated by insect pollinators (Carew *et al.*, 2003). Therefore, despite their capability for self-pollination, insect visits are essential for ensuring complete strawberry set.

According to Free (2015), the deformation rate of strawberries is about 48.6% in the absence of pollinating insects. Insect visits have been found to exhibit a significant effect on an increased percentage of well-formed and bigger-sized fruits (Forrest and MacInnis, 2019). Moreover, Rajan *et al.* (2020) highlighted that ineffective pollination and lack of adequate pollinator species constrain strawberry production. Overall, this research aims to fill the knowledge gap regarding the diversity of pollinators species in dry subtropical region of Punakha and their effect on Camarosa, strawberry yield and quality, with potential implications for sustainable strawberry production practices.

MATERIALS AND METHODS

Experimental Design and Plot Preparation

The experiment was conducted at the College of Natural Resources under Royal University of Bhutan, Lobesa (27°30'11" N and 89°52'42" E) from December 2021 to June 2022. The site stands at 1,440 masl and experiences sub-tropical climate. There were two treatments, namely, T_1 - Open plots of strawberry and T_2 - Mesh screen caged plot as a control. The experiment was arranged in a randomized complete block design with four replications per treatment. Each replication plot was 4 m x 1 m and accommodated 18 plants (144 in total). One-year old healthy runners of selected cultivar Camarosa at 4-5 true leaf stage were transplanted in well-prepared plots, which were raised uniformly to about 12 cm above the ground. Plants were planted with row to row and plant to plant spacing of 45 cm each. A space of 1 m between the two plots was

maintained. The control set up was covered with a mesh screen of 0.9 mm hole to exclude potential pollinators.

Sampling Method

Observation of pollinator insects visiting the flowers was recorded to analyze species diversity and abundance by following two methods namely, opportunistic visual encounter and observational plot method. Cone type hand net was used for collecting the insect specimens. The netting of cone type hand net was standardized from 6 a.m. to 5 p.m. to ensure sampling of the total flying community occurred. The captured insects were placed in a container containing a cotton soaked in 70% ethanol.

The collected pollinator species specimens were pinned, dried and stored in the specimen box. Taxonomic identification of bee species was done following keys and descriptions provided by Engel (2001a, 2002b and 2012c) and Dorji *et al.* (2016). Similarly, ant species was identified using Dendup *et al.* (2021) preliminary checklist of ants from Bhutan and butterfly species by following Mehra *et al.* (2018) keys and description. In addition, the sampling of strawberry plant was done through complete enumeration sampling method. The data for fruit yield and quality attributes were collected twice until the fruiting of strawberry ceased.

Observations Recorded

Pollinator diversity: The diversity of pollinator species visiting strawberry blossom, their relative abundance, species richness and evenness were recorded. Visual counts of insect visitors were made in the early morning (6 a.m. to 9 a.m.), late morning (10 a.m. to 11 a.m.), noon (12 p.m.), early afternoon (12 p.m. to 3 p.m.), late afternoon (3 p.m. to 5 p.m.) and evening (5 p.m. to 7 p.m.).

Fruit yield parameter: Number of fruits per plant, number of fertilized achenes per fruit, fruit diameter and total fruit weight were recorded. Fruit weight and fruit diameter were determined using an electric weighing balance and a digital vernier calliper, respectively.

Fruit quality parameter: Fruits from both the treatments were tested for their total soluble solids (TSS), color and shape after harvest (Bagnara and Vincent, 2015). TSS (%) of fruits were tested using digital ^oBrix refractometer on the same day of fruit harvest. The grading of fruit color was done using a horticulture color grading chart, Fan 1 (Red group) that categories the pigments to grade A, B and C. Grade A signifies the best color of the fruit with dark deep red color followed by B and C as inferior fruit color. The fruit shape was assessed using fruit deformation scores, that is recorded as: 1 - badly misshapen and unmarketable quality; 2 - somewhat malformed between 1 and 3; and 3 – perfectly symmetrical and marketable quality.

Weather parameter: The temperature and relative humidity were recorded using a digital data logger to determine their association to the population dynamics of insects visit in strawberry. In order to determine the diurnal variation in pollinator numbers visit during strawberry flowering, days were defined into morning (6 a.m.-noon), midmorning (8-10 a.m.), afternoon (noon-4 p.m.), mid-afternoon (2-4 p.m.), and evening 4-6 p.m.). During winter, morning officially begins at dawn (5 a.m.), and ends at noon (12 p.m.). As the name suggests, afternoon begins at 12 p.m. and continues for several hours till 4 p.m. Evening is that time when the sunlight starts to fade and ends once darkness settles in. They were defined based on sun's patterns during winter with reference to Lin (2021).

Data Analysis

All the data were analyzed using the statistical software R version 2.13.1. Descriptive statistics were used to assess the total number of pollinator species recorded during flowering in strawberry. Shannon diversity index was calculated using the following formulae: $H=\sum[(pi)\times ln(pi)]$. where pi is the proportion of individuals belonging to the ith species. Two sample t-test was used to test the impact of pollination on the strawberry yield parameters and fruit quality attributes. Treatment means with a probability threshold of p < 0.05were considered statistically significant. The influence of climatic conditions particularly the temperature and relative humidity on pollinators' visit was compared using Pearson's correlation analysis.

RESULTS AND DISCUSSION

Diversity of Insect Pollinators Visiting Strawberry Bloom

Overall, a total of 840 insect pollinators belonging to six species under two orders and three families were observed (Table 01). The most dominant pollinators recorded were *Tetramorium* sp. (ant), (n = 506, RA = 60.24%) followed by *Apis cerana* (n = 235, RA = 27.9%) and the least dominant *Junonia almana* (butterfly), (n = 2, R_A = 0.24%). The overall species diversity of the area was H = 1.03, species evenness $E_H = 0.58$ and species richness $S_R = 1.71$. The other insect visitors included grasshoppers, crickets, aphids, cutworms and houseflies. The latter insect groups visited the strawberry bloom at interrupted intervals and some insects such as aphids and cutworms caused damage to plants. Therefore, they could rather be considered as a pest and unreliable pollinators for strawberry pollination.

The visiting density of *Tetramorium* sp. is highest in strawberry and it's observed that this insect is an effective pollinator. Although ants in general were often considered as a generalist visitor in flowering plants, a study by Delnevo et al. (2020) reported ants as effective pollinator in Conospermum undulatum plant. Tetramorium ants are also recorded to be essential for the pollination of Cytinus hypocistis (Vega et al., 2009). These insects are equally important pollinator source for strawberry due to their long foraging range being active during a larger part of the day and ability to pollinate in varied weather conditions (Figure 02). This makes them potentially reliable pollinators under unstable weather conditions as well (Heinrich, 2004).

This study recording the diversity of insect pollinators species and their effect in strawberry production is the first of its kind in Bhutan. The study found that *Apis cerana* was the second predominant floral visitor. It was observed that the presence of *Robus ellipticus* plants that belong to the same family (Rosaceae) of strawberries, served as both foraging resources for *Apis cerana*. Moreover, the flowering period of strawberry coincided with the onset of flowering of *Robus ellipticus*, which were growing in close vicinity to the trial site, potentially attracting *Apis cerana* to visit strawberry plants as well.

Table 01: Overall	pollinator spe	cies composition	visiting during	strawberry blossom
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Order	Family	Pollinator Insect	п	RA (%)
Hymenoptera	Apidae	Apis cerana	235	27.9
	Apidae	Apis laboriosa	52	6.1
	Apidae	Xylocopa acutipennis	9	1.07
	Formicidae	Tetramorium sp.	506	60.2
	Apidae	Bombus sp.	36	4.2
Lepidoptera	Nymphalidae	Junonia almanac	2	0.2

Shannon diversity index = 1.03, species evenness = 0.58, Species richness = 1.71



Figure 02: Pollinators' visits in relation to daily diurnal variation.

Other wild plants in the vicinity of the trial site such as *Desmodium elegans* and *Caselpinia decapetala* attracted larger-sized bee species like *Xylocopa acutipennis* and *Bombus* sp. Wild plants growing close to the trial site represented a valuable refuge area for pollinator insects in the study. However, there may be annual and regional variances for the visit of these pollinator species during flowering, depending both on field margins found in the vicinity of the strawberry fields and abiotic factors like temperature, rainfall and wind velocity.

According to Klatt *et al.* (2014a), bees are by far the main strawberry flower pollinators and account for 98.5% of the insect pollinators. It was previously reported that *Apis mellifera* and *Apis cerana* are worthy strawberry pollinators (Abrol *et al.*, 2017). In Udheywala Jammu, bees belonging to apidae including *Apis mellifera*, *Apis cerana, Apis florea,* and *Apis dorsata* were listed as common visitors to strawberry flowers (Abrol *et al.*, 2017). However, in this study, the co-occurrence of insects of different sizes and types are reported to impact the pollination system in strawberry.

Foraging Behavior of Pollinating Insects

The study found differences in foraging behavior of different insect pollinators in strawberry flower. The bee species, Apis cerana, Apis laboriosa, Xylocopa acutipennis Smith, and Bombus sp. always landed on the top of flowers and occasionally on the petal zone. During visits, Apis cerana and Apis laboriosa were observed performing a circular movement alternating between pollen and pistils. Whereas, the bumble and carpenter bees (Xylocopa acutipennis) were found to be more aggressive, collecting all the nectars and as such in a small crop setting, they could be expected to cause damage to the flower. A lone butterfly species, Junonia almana in the study site ensured contact with head to the flower as its proboscis dug in to reach for nectar, while bee species made the contact using their whole body. Tetramorium sp. due to their smaller body size, restricted most of their action to the basal zone of pistils.

Biotic Pollination Influence

Pollination influence on achene formation: Limitation in strawberry pollination is determined by the number of fertilized achenes and distribution of achenes on the surface of the receptacle (Cekic et al., 2018). In plots deprived of insect pollinators such as in the cage, number of achenes was fewer and also there was a presence of an aggregated small unfertilized achenes at the deformed area of fruits. A two-sample t-test showed a significant difference in the number of fertilized achenes between open plots (174 \pm 2.94) and caged plots (167 ± 3.16) ; $t_{(428)} = 18.302$, p < .001 (Table 02). Such deformation in fruits were the result of either unfertilized achenes or missing achenes owing to poor pollination condition (Klatt et al., 2014b) corroborating the efficacy of pollinators in pollination.

Pollination influence on fruit quality and yield parameters: A two-sample t-test showed that there was significant influence of pollination due to insects on number of fruit sets per plant between open and caged plots, t $_{(142)} = 2.57$, p = .011. The fruit number per plant in the open plots were higher (M = 18.43) than in caged plots (Table 04). Similarly, the average weight of the fruits recorded from caged plots was significantly lower (M = 10.93) than the average weight of strawberry fruits produced in the open plots (M = 14.4). The results further showed that there was a statistically significant difference between open and caged plots in terms of fruit diameter at t (428) = 12.856, p < .001 with a mean rank of M = 31.12 mm in the open plots compared to caged plots at M = 26.95 mm. The average TSS (^obrix) of fruits grown in open plots was slightly higher (M = 7.66) than the TSS (^obrix) of those fruits growing in the caged plots (M = 6.20). The occurrence of these varied fruit qualities and yield effects in open and caged plots are driven by the release of growth hormone such as auxin in open plots as a result of pollination process (Klatt et al., 2013a). It's reported that auxin coordinates achene and receptacle formation in strawberry fruits (Tian et al., 2022).

The study found that the exclusion of pollinators reduced the number of fruits set and delayed fruit maturity. Moreover, lack of pollination produced smaller-sized and light weight fruits with reduced fruit diameter compared to strawberries grown in open plots where diverse pollinator insects were present. However, the direct effect of lack of pollinators and pollination was on the shape of the fruits produced as they were deformed. Previous studies on strawberry fruit deformation were not completely explained by differences in open and caged plots, neither by differences in achene formation and arrangement on receptacle, suggesting that fertilized achenes and their arrangement are as a result of pollination by pollinator insects which might affect the fruit shape.

Table 02:	Descriptive statistics for 1	number of fertilized achene	es between open and cage plot.
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Plot types	Ν	М	SD	Min	Max
Open plot	215	174	2.94	107	210
Caged plot	215	167	3.16	78	180

Table 03: Q	uantitative yield	parameter of both o	pen and mesh screen	cage plots
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Treatments	No. of fruits	Fruit diameter (mm)	Fruit wt. (g)	TSS (^o Brix)
Open plot	18.43±4.8	31.12±3.36	14.4±4.14	7.66±2.057
Caged plot	16.72±2.95	26.95±3.35	10.93 ± 3.55	6.20±1.765

Treatments	Color grading			Fruit deformation score		
	A (%)	B (%)	C (%)	1 (%)	2 (%)	3 (%)
Open plots	86	14	0.0	81.2	12.8	6
Caged plots	62.3	29.3	8.4	52.8	45	26.6

Table 04: Quality parameters of fruits grown in open plots and caged condition.

Grade A signifies the best color of the fruit followed by B and C as low. 1-Perfectly symmetrical and marketable quality, 2-somewhat deformed between 1 and 3-badly misshapen and unmarketable.

The grading of fruit color was studied using a horticulture color grading chart, Fan 1 (Red group) that categorizes the pigments into grades A, B and C. Grade A signifies the best color of the fruit followed by B and C as low. The results showed that the best fruit color was obtained from the open plots with a maximum number of fruits with grade A (86%). Whereas, the fruit color obtained from caged plots was inferior and only 62.3% of fruits were of grade A color. Moreover, the caged plots contained 29.3% grade B color and 8.4% grade C color (Table 05).

The deformation score of fruits resulting from flowers foraged by pollinator insects in open plots differed significantly from the caged plots. The fruits of perfectly symmetrical and marketable quality were more in the open plots (81.2 %) than in caged plots (52.8 %). Fruit deformation score (particularly badly misshapen and unmarketable fruits) was found to be the highest from caged plots at 26.6 %. However, in open plots the fruits with badly misshapen and unmarketable quality were recorded to be only 6 % (Table 05).

Bigey *et al.* (2005) had reported that malformed and smaller-sized strawberry fruits developed in poor pollination condition. Strawberries grown in open plots produced fruits with a greater number of fertilized achenes and development of such achenes are a result of successful and complete pollination (Klatt *et al.*, 2014b). Ultimately, successful pollination leads to fruits with larger size and diameter. Achenes containing fertilized ovules release an auxin hormone that stimulates enlargement of the receptacle, which enhance the size of fruits (Tian *et al.*, 2022). When an achene does not contain a fertilized seed, it remains small and the receptacle in its area restricts enlargement. Therefore, when a group of such unfertilized achenes occur together the fruit gets noticeably deformed and inferior in quality.

The results of the study highlight that pollination affects the fruit characteristics of strawberry like fruit mass, shape, color and taste attributes such as ^oBrix content. Dung *et al.* (2021) in their research iterated that flavor attributes are regulated by paternity of the seeds and pollen source and not by number of fertilized seeds per fruit. However, comparative study of this research between open and caged plots found that ^oBrix content of strawberry is rather greatly influenced by pollination process.

Association Between Climatic Conditions and Pollinators Visit

Diurnal variation of pollinator insects with respect to different time of the day: The highest number of insect pollinators were recorded in early afternoon (n = 184) during the driest part of the day. The most abundant group of insect pollinators recorded during early afternoon belonged to the order Hymenoptera (99%) and the least to order Lepidoptera (1.1%). Tetramorium sp. was the most abundant pollinator throughout the day. The least visit of insects was recorded during evening with a total of 93 insects (Figure 02).

The visit of insects during strawberry blossom differed during different times of the day. Pollinators in the study site exhibited enhanced foraging activity early afternoon (12 p.m. to 3:00 p.m.) when nectar was at its daily peak of availability (Zhang *et al.*, 2019). Moreover, pollen availability in the flower reaches its maximum in afternoon during the driest part of the day (Najberek *et al.*, 2021). In the early morning and late evening hours, when it is cooler, air humidity and condensation on the flowers and leaves make the pollen sticky, thus, restricting the dispersal of pollens to initiate pollination process (Iovane *et al.*, 2022).

Subsequently, the fewer visit of pollinators in strawberry blossom. Viability of pollen and amount of pollen available changes in flowering plants throughout the day (Zhang *et al.*, 2019). Thus, insects are expected to visit flowers during the most favorable conditions to ensure a successful pollination. Through the empirical evidence from the study, the best time for insect visit for pollination was found to be between one to four days after flower opening and before the petal falling and pistils beginning to dry.

Relation between climatic conditions and pollinators visit: On clear and sunny days, at a minimum temperature range of $11^{\circ}C-14^{\circ}C$, least number of pollinators were recorded (n = 26). The number of pollinators began to increase sharply from $15^{\circ}C$ untill the temperature reached 27°C. The highest number of pollinators (n = 108) was recorded between $23^{\circ}C-27^{\circ}C$. The fall in temperature to $19^{\circ}C-22^{\circ}C$ also saw corresponding decrease in pollinators numbers (n = 104) (Figure 03).

Foraging populations of *Bombus* sp. was negatively influenced by relative humidity. Each insect pollinator is reported to have specific temperature and relative humidity threshold for their foraging activity (Abrol *et al.*, 2017). Although the relative humidity on its own does not fluctuate the foraging activity of pollinators, the combination of temperature and humidity greatly influenced their visit. Low temperatures (11-14°C) and high humidity (above 70%) had the double effect of reducing foraging activity of pollinators. Likewise, high temperature (above 28°C) and low relative humidity (20-40%) also had minimum pollinator visits (Figure 03 and Figure 04).

Overall, with increasing temperature from 11°C to 27°C together with the optimum relative humidity of 51% to 70% during day time subsequently, increased the pollinators visit to the strawberry blossom. On the other hand, at 28°C and above, the pollinator visit recorded was the least (n = 6) throughout the study period. The difference in their visits was caused by the favorable condition for pollen release at a temperature of about 20°C and humidity of 70% or less (Abrol *et al.*, 2017). The data clearly revealed that each insect pollinator had its specific environmental threshold for their field activities.



Figure 03: Total number of pollinators visit in relation to temperature.



Figure 04: Insect pollinators population with respect to relative humidity (%).

CONCLUSIONS

Insect pollinators in strawberry is crucial for successful pollination and to enhance fruit productivity. Through current study, it has shown that diverse range of pollinator species, including bees, butterflies and ants affected strawberry in ensuring better productivity and quality. Understanding the diverse community of insect pollinators in strawberry would enable strawberry growers to understand the need for maintaining and enhancing pollinator species in strawberry fields. This study acknowledges the contribution of pollinator species in strawberry production and highlights on enhancing the sustainable strawberry farming practices through pollination as one main component.

The study results portray that insect pollinators should be acknowledged for their contribution in improving the fruit marketable qualities and productivity. They should be recognized for their role in initiating the release of plant hormones that poses significant role in determining the crop yield and quality. The study suggests farmers and strawberry growers to cultivate strawberries in the open field to provide pollination services rather than growing inside the protected environment such as greenhouse. In addition, study suggest strawberry growers that early afternoon (12 p.m. to 3:00 p.m.) as the best time to open the greenhouses to allow insect pollinators to visit flowers. Furthermore, the result highlights the importance of maintaining field margins that notably serves as a foraging resource and nesting site to attract pollinators.

Effects of lack of pollination on deformed fruits shape, lower yield and poor fruit quality underscores the importance of pollination in strawberry production. Moreover, empirical evidences from the study shows the need to consider pollination services in strawberry

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where, pollination services are in danger of various anthropogenic threats including forest degradation, climate change, pesticide use in fields and so on. This study conveys, managing insect pollinators on strawberry farms as an important factor to ensure the best possible fruit quality and strawberries yield success. Overall, it can be concluded from this study that the diversity of pollinator species, their effect to the productivity and quality of fruits are closely related.

The results obtained in this research indicated that pollinator species exhibit important impact on strawberry productivity through pollination process. These diverse range of pollinator species as observed in the present study belongs to bees, butterfly and ants. To maximize the fruit yield and quality of strawberry, it is important to adopt measures that promote and protect these key pollinators, such as providing suitable habitats and minimizing pesticide use. Additionally, future research should focus on exploring strategies and methods to enhance pollinator populations and their effectiveness in strawberry cultivation, ultimately benefiting both farmers and consumers.

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