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Refugia flowers enhance insect pollinator and quality of fruit production in strawberry farming

SAKINAH AMANINA[1], NINA MARYANA[2,♥], I WAYAN WINASA[2]
[1]Program of Entomology, Graduate School, Institut Pertanian Bogor. Jl. Kamper Wing 7 Level 5, Kampus Dramaga, Bogor 16680, West Java, Indonesia
[2]Department of Plant Protection, Faculty of Agriculture, Institut Pertanian Bogor. Jl. Kamper, Kampus Dramaga, Bogor 16680, West Java, Indonesia.
Tel.: +62-251-8629364, Fax.: +62-251-8629362, ♥email: ninama@apps.ipb.ac.id

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Abstract. *Amanina S, Maryana N, Winasa IW. 2025. Refugia flowers enhance insect pollinator and quality of fruit production in strawberry farming. Biodiversitas 26: 1310-1316.* Strawberry flowers have different receptive and anthesis periods, making them generally cross-pollinated. Adding refugia flowers in strawberry farming can attract more insect pollinators, enhancing successful cross-pollination and complete fruit formation. This research aimed to study the effect of refugia flowers in improving the diversity and abundance of insect pollinators and, eventually, fruit production and quality in strawberry farming. The research was conducted in Sukaresmi Village, Rancabali Sub-district, Bandung District, West Java, Indonesia using a randomized group experimental design consisted of four treatments, namely strawberries plants without refugia (control/C), strawberries and common zinnia (*Zinnia elegans*) (SZ), strawberries and marigold flowers (*Tagetes* sp.) (SM) and strawberries and cosmos flowers (*Cosmos* sp.) (SC). Observations of pollinators were conducted at 06.00-09.00, 10.00-13.00, and 14.00-17.00 every three days for 30 days. Data was subjected to analysis of diversity and abundance, Analysis of Variance (ANOVA), Chi-Square test, and correlation analysis. The highest pollinator diversity was found in the SC treatment, and the lowest was in the control, although the diversity level in all treatments was moderate. The cosmos flowers also attracted the most significant number of individual pollinators. Three dominant pollinators were found in all plots, i.e. *Apis cerana*, *Melanostoma* sp., and *Episyrphus viridaureus*. Furthermore, the abundance of pollinators affected the quantity of perfect fruit and quality of strawberry fruits, with SC treatment producing the highest fruit yield and quality on average. The findings of this study imply that adding cosmos flowers positively impacts pollinators, which helps produce better strawberries.

Keywords: Attractant, field modification, malformation, pollination

INTRODUCTION

Strawberries are very popular commercial fruit, sold directly for fresh consumption and processed into foods (e.g., jams and jellies) and beverages. More recently, this fruit has also increasingly been utilized as a tourist attraction, such as strawberry picking (Prasad et al. 2022). Although this fruit is native to temperate regions of the northern hemisphere, there is increasing development of strawberry cultivation in tropical countries, including Indonesia. For example, there was an increase in strawberry production in West Java every year from 2019 to 2022 (BPS 2023). In 2019, strawberry production was only 4,758 tons, which increased in 2020 to 5,955 tons; in 2021, the production further increased to 6,458 tons, and in 2022, the production increased dramatically to 25,413 tons. This data shows the increasing interest of farmers in cultivating strawberries as a source of income because the demand for strawberries increases significantly.

Strawberries have hermaphroditic flowers with different receptive and anthesis periods. Female reproductive organs are receptive earlier to experience anthesis than male reproductive organs, so strawberries generally experience cross-pollination (Miranda et al. 2024). Self-pollination in strawberries is still possible, although it is generally more dominated by cross-pollination for successful fruit formation (Dorji et al. 2023). Insect pollinators in highly diverse and abundant crops contribute to fruit formation through successful pollination (Ahrenfeldt et al. 2015). The research by Masyitah et al. (2019) showed that pollinators, such as bees and flower flies, present in the planting area affect the quality and quantity of strawberry crop yields. Strawberry flowers pollinated by pollinators have more fully formed fruits, a percentage of flowers developing into fruit, and yield quality value in weight, length, and diameter. The most effective insect pollinators of strawberry flowers are from the bee group, including *Apis cerana* and *Tetragonula laeviceps*, which can increase strawberry fruit production when environmental conditions support pollination (Alpionita et al. 2021). Other bee species that visit strawberry flowers in the field when the strawberry flowers are in full bloom are *A. mellifera*, *A. florea*, *A. dorsata*, *Andrena leaena*, *Halictus* sp.. In addition to bees, other pollinators of strawberry flowers are from the fly group, i.e. *Episyrphus balteatus* and *Melanostoma univittatum* (Abrol et al. 2017).

Increasing the diversity and abundance of pollinators can be pursued in several ways, one of which is modifying the environment where the plant is growing, one of which is by planting other flowering plants to serve as refugia. Refugia plants are essential in promoting insect conservation to maintain agroecosystem balance (Windriyanti et al. 2023). Besides attracting pollinators, refugia flowering plants also attract natural enemies and play a role in regulating pest populations, crop pollination, and maintaining biodiversity (Broadley et al. 2022; Fei et al. 2023; Kulkarni et al. 2023). Some examples of refugia plants include *Zinnia elegans*, *Tagetes* sp., and *Cosmos* sp., which can also be used as ornamental plants in the yard. The attractive properties possessed by the flowers of refugia plants (e.g. color, shape, and scent), and the content of nectar and pollen which serve as food sources can stimulate pollinators to visit the flowers (Chen et al. 2023).

In addition, the compound contained in the flowers of refugia plants also attracts pollinators. The research by Fadhilah (2023) revealed that marigold flowers and common zinnia have titanium dioxide and cellulose compounds. Titanium dioxide compounds can attract insects by releasing carbon dioxide and heat effects, so volatile compounds like cellulose-containing phenols in plants can spread quickly in the cultivation area (Okto and Munasir 2023). Another factor that attracts insects is the presence of sugar, as in the case of cosmos flowers since these flowers have an average concentration of sugar content in nectar of not less than 31%, which is helpful to fulfill the needs for food and honey for the pollinators (Biroki and Tchuenguem 2021).

This study aimed to investigate the effect of refugia flowers on the diversity and abundance of pollinating insects in strawberry cultivation in Rancabali Sub-district, Bandung District, West Java Province, Indonesia. The study also analyzed the correlation between the presence of refugia flowers and strawberry crop production. We used common zinnia (*Z. elegans*), marigold flowers (*Tagetes* sp.), and cosmos flowers (*Cosmos* sp.) as refugia plants to attract the presence of pollinators and to determine the type of refugia that most effectively attracts pollinators. Adding refugia flowers to strawberry cultivation can have a positive impact on increasing pollinators and helping pollination of strawberry flowers so that strawberry fruit production is optimized.

MATERIALS AND METHODS

Study period and area

This research was conducted from March to September 2024 in Sukaresmi Village, Rancabali Sub-district, Bandung District, West Java, Indonesia. Insect pollinators were identified at the Insect Biosystematics Laboratory of the Department of Plant Protection, Faculty of Agriculture, Bogor Agricultural University.

Research materials and design

This study used the strawberry cultivar of *mencir*. This study used a randomized group design because all observation plots have a wide expanse in the same location. We modified the planting configuration by adding refugia flowering plants, which were inserted two weeks after planting (Figure 1). There were four treatments of planting configuration in this study, namely SZ (strawberries and common zinnia), SM (strawberries and marigold flowers), SC (strawberries and cosmos flowers) and C (strawberries without refugia) which served as control. The distance between plots treated with refugia flowers was 40 m, while between the treated plots and the control plot was 80 m.

Each plot was sampled using systematic diagonal sampling, which resulted in five subplots (Figure 1). Each subplot contained 12 polybags of strawberry plants, and each bag contained four strawberry plants. Thus, the total sample plants for one plot were 240 strawberry plants (Figure 1.A). In the treated plots, there were additional refugia flowering plants, as many as 25 polybags, with each subplot containing five polybags of refugia flowers (Figure 1.B).

Data collection

Tagging strawberry flower

The flowers of the sampled strawberry plants that began to bloom were marked using labels. Pollinators were observed from the blooming until the first harvest, which was about one month. The mark assigned to the strawberry flowers aimed to limit the strawberry fruits harvested as sample data.

Observation of insect pollinators diversity

Observations of pollinating insects were conducted every three days for 30 days. Each observation was conducted in three different periods, i.e. 06.00-09.00, 10.00-13.00, and 14.00-17.00. Pollinators visiting strawberry flowers and refugia flowers were collected using insect nets or plastic, then preserved dry (pinnin*g*) and wet in vials bottles with 70% ethanol.

Observation environmental conditions

Environmental conditions observed included temperature and humidity using a thermo-hygrometer, wind speed using an anemometer, and light intensity observations using a lux meter. Measurement of environmental conditions was carried out every three days during pollinators observation. Environmental conditions have an impact on pollinators activity (Atmowidi et al. 2022).

Strawberry harvesting

Strawberry harvesting was conducted from 80-120 days after planting. The fruit quantity and quality from each subplot were recorded during the harvesting. Yield quantity was measured based on the number of fully formed fruits, while fruit quality included the fruit's weight, length, and diameter. Fruit weight was measured using a digital scale, while fruit length and diameter were measured with a ruler. The fruit quality assessment was divided into five categories, as presented in Table 1.

Table 1. The classification of strawberry fruit quality based on weight, diameter, and length

Category	Weight (g)	Diameter (cm)	Length (cm)
A	$X > 11$	$Y > 3.2$	$Z > 3.2$
B	$9 < X \leq 11$	$2.7 < Y \leq 3$	$2.5 < Z \leq 3$
C	$6.5 < X \leq 9$	$2.1 < Y \leq 2.9$	$2.5 < Z \leq 3$
D	$3 < X \leq 6.5$	$2.0 < Y \leq 2.9$	$1.8 < Z \leq 2.4$
E	$X \leq 3$	$Y \leq 2$	$Z \leq 1.8$

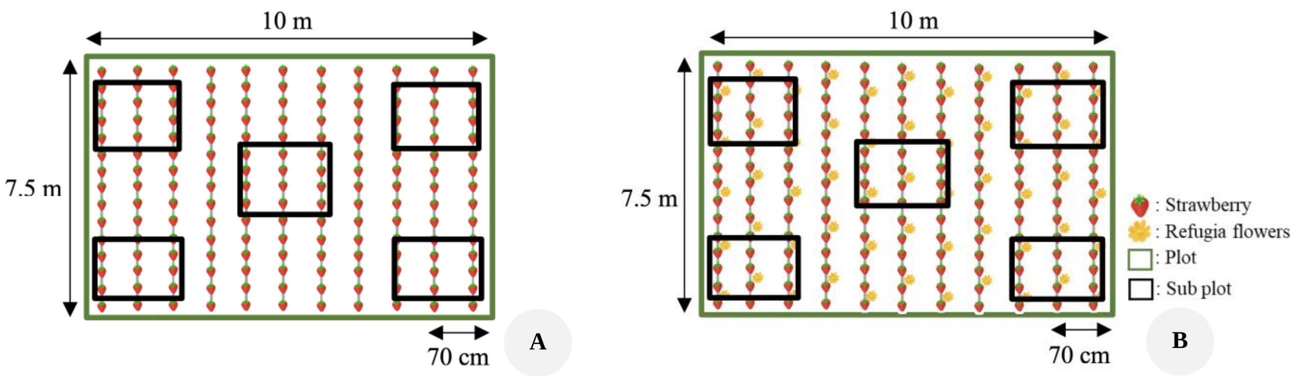

Figure 1. The diagram of experimental design shows: A. The control plot; B. Treated plots

Insect identification

Insects from the observations were identified to the family and morphospecies level. The identification was carried out using the identification key by Vockeroth and Thompson (1992), Goulet and Huber (1993), Michener (2007), Nazarreta et al. (2021), Panjaitan et al. (2021), and other publications.

Data analysis

Data was analyzed by measuring pollinators diversity using the Shannon-Wienner index formula (H'), species evenness using the Evenness index (E), and species dominance using the Simpson index formula (D). In addition, an analysis of variance was also conducted to determine the effect of refugia flowers on pollinator diversity and the effect of pollinator abundance on strawberry fruit quality. The statistical significance was tested using Tukey's test with a level of 5%. Chi-Square test was also conducted to determine the distribution of pollinators abundance during observation. Correlation analysis determined the relationship between pollinators abundance and yield quantity. All data results were tabulated in Microsoft Excel software. The process of analyzing the research data was carried out using R-Studio software. The analysis results were presented as boxplots, diagrams, and tables.

RESULTS AND DISCUSSION

Diversity and abundance of insect pollinators

Based on the observation results, four orders of insect pollinators were found in the four observation plots: Hymenoptera, Diptera, Coleoptera, and Lepidoptera, with Hymenoptera having the highest percentage of species. This result is in line with the research conducted by Aldini et al. (2019) that Hymenoptera had the most species found in the strawberries planted along with cosmos, common zinnia, and marigold as refugia flowers. There were 28 species from 12 families with three species being dominant in all observation sites (Table 2). Hymenoptera order had one species with the highest number of individuals across observation sites, i.e. *A. cerana* with 669 individuals. The second and third dominant species were from the order Diptera, i.e. *Melanostoma* sp. with 529 individuals and *E. viridaureus* with 414 individuals. According to Rader et al. (2016), bees act as potential pollinators for crops, although other species can serve similar roles such as flies. Flies from the family Syrphidae (hoverflies) are examples of insects that can be effective pollinators. Syrphidae family visitation in strawberry cultivation can increase crop yield by up to 70% (Hodgkiss et al. 2018). Therefore, the presence of three dominant species in this study was advantageous for helping strawberry plants pollinate.

The highest number of individuals and species of insects was found in the strawberry and cosmos flowers treatment (SC) with 21 species and 1031 individuals, followed by strawberry and common zinnia (SZ) with 20 species and 886 individuals and strawberries and marigold flowers (SM) with 13 species and 700 individuals (Table 2 and Figure 2). The lowest number of individuals and species was at the control treatment (C) with 571 individuals. This finding suggests that the variety of refugia plants in strawberry farming positively impacts insect diversity and abundance.

The diversity index of pollinators in strawberry farming in all treatments is in the medium category since the value is between 1 and 3, while evenness has a low category because the value is less than 0.4, and dominance is in the high category because it is between 0.75 and 1.00 (Table 3). The four treatments have similar diversity categories since the number of species is moderated by the number of individuals in each treatment (Karenina et al. 2019). The evenness and the dominance have an inversely proportional relationship, if few species are dominant, then the distribution of insects tends to be uneven, and vice versa. The low evenness category indicates that the abundance of pollinators is not uniform for all treatments. Meanwhile, the high dominance category shows that all treatments are dominated by only a few species, particularly by *A. cerana*, *Melanostoma* sp., and *E. viridaureus*.

The species richness of pollinators was analyzed using variance analysis. The analysis showed that different treatments significantly affected the species' richness ($F_{3,12}$: 11.361; P: 0.0008). The boxplot in Figure 3 shows that the strawberry+common zinnia treatment (SZ) and strawberry+cosmos treatment (SC) are very significantly different from the control treatment, while the strawberry+marigold treatment (SM) and control treatment are different but not significant. Several morphological factors in each refugia flower can cause this. Marigold flowers are lower than the cosmos and common zinnia. The height of marigold flowers does not exceed the height of the strawberry, while the height of cosmos and common zinnia flowers is higher than those of strawberry. Wróblewska et al. (2016) also argued that marigold flowers have a pollen size of 39 μm, while common zinnia has a smaller pollen size of 14.05 μm and cosmos flowers have a size of 32.63 μm. The large pollen in marigold flowers might attract pollinators to be adapted to the size of their mouth apparatus.

Table 2. Number of species and individuals of insect pollinators in each treatment

Order: Family / Morphospecies	C	SZ	SM	SC	Total
Coleoptera: Scarabaeidae					
Popillia japonica	12	23	14	28	77
Diptera: Syrphidae					
Episyrphus viridaureus	92	105	102	115	414
Melanostoma sp.	105	140	117	167	529
Hymenoptera: Apidae					
Amegilla cingulata	0	4	0	3	7
Apis cerana	141	174	156	198	669
Apis dorsata	0	14	0	58	72
Ceratina sp.	17	44	32	60	153
Nomada sp1.	0	0	0	11	11
Nomada sp2.	0	0	0	9	9
Tetragonula laeviceps	57	74	68	82	281
Thyreus nitidulus	0	5	0	3	8
Hymenoptera: Crabronidae					
Craboninae sp.	0	0	0	2	2
Hymenoptera: Halictidae					
Halictus sp.	43	73	60	78	254
Lasiglossum sp.	56	79	63	77	275
Hymenoptera: Ichneumonidae					
Glabridorsum stokesii	0	0	0	8	8
Hymenoptera: Scoliidae					
Campsomeriella annulata	42	75	68	82	267
Megascolia azurea	0	0	2	0	2
Phalerimeris phalerata	0	0	0	14	14
Scolia rugifrons	0	0	2	0	2
Hymenoptera: Sphecidae					
Sceliphron spirifex	0	0	0	5	5
Hymenoptera: Vespidae					
Vespa velutina	0	10	6	13	29
Lepidoptera: Erebidae	6	66	10	19	101
Amata huebneri	0	4	0	0	4
Nyctemera adversata	6	20	10	16	52
Lepidoptera: Nymphalidae					
Melantis leda	0	6	0	3	9
Symbrenthia hypselis	0	2	0	0	2
Vanessa cardui	0	3	0	0	3
Lepidoptera: Papilionidae					
Graphium sarpedon	0	14	0	0	14
Papilio memnon	0	17	0	0	17
Number of species	10	20	13	21	

Note: C: Strawberry/control; SZ: Strawberry+common zinnia; SM: Strawberry+marigold; SC: Strawberry+cosmos

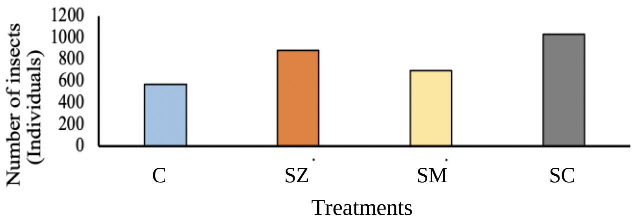

Figure 2. Abundance of insect pollinators in each treatment. Note: C: control treatment, SZ: strawberry+common zinnia treatment, SM: strawberry+marigold treatment, SC: strawberry+ cosmos treatment

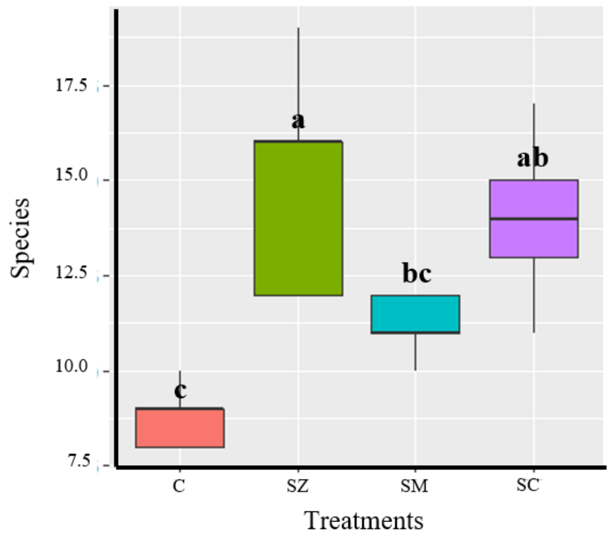

Figure 3. Species richness of pollinating insects in each treatment. C: Control; SZ: Strawberry+common zinnia; SM: Strawberry+marigold; SC: Strawberry+cosmos. Boxplots with different letters indicate differences according to the Tukey test (P<0.05)

Abundance of insect pollinators based on observation time

Observations of pollinator insects conducted at three different times are related to environmental conditions. Environmental conditions such as temperature, humidity, wind speed, and light intensity influence the presence of the insect population since these factors might facilitate the insects to move. Based on the observations, pollinators were the highest in the morning, began to decline in the daylight, and increased again but not significantly in the afternoon (Figure 4). The result analysis showed a significant difference in insects' abundance at each observation time (χ^2: 660, 54; p: $3.67x10^{-144}$). The result is similar to Sinaga et al. (2024), who reported that pollinators are found mostly in the morning and decrease during daylight. The difference in insects' abundance regarding time is related to the availability of nectar and pollen. Descamps et al. (2021) reported that climate can directly affect insects visiting flowers, decreasing the nectar volume and sucrose amount that plants offer. Tschoeke et al. (2015) stated that the abundance of *A. cerana* as the most common pollinators was high in the morning due to increased pollen and nectar availability, which decreased in the afternoon.

The effect of refugia flowers on fruit production

Insect pollinators affect strawberry production. Incomplete pollination can cause strawberry fruit to become malformed (Figure 4). The abundance of pollinators was analyzed to determine whether the abundance of pollinators affects fruit quality (Table 4). Based on the results of the variance analysis, insect abundance significantly influenced fruit length ($F_{3,12}$: 8.249; P: 0.003) but not fruit diameter ($F_{3,12}$: 2.114; P: 0.152). The fruit quality parameter significantly associated with pollinators abundance was strawberry fruit weight ($F3_{,12}$: 30.82; P: $6.4x10^{-6}$). Cao et al. (2023) reported that pollination of strawberries with honey bees not only helps to increase crop yield but also improves the quality of strawberries because it is related to the acceptance of strawberry stigmas that are more optimal. This statement is in line with our study's observation where honey bee species were found, namely *A. cerana* and *A. dorsata*.

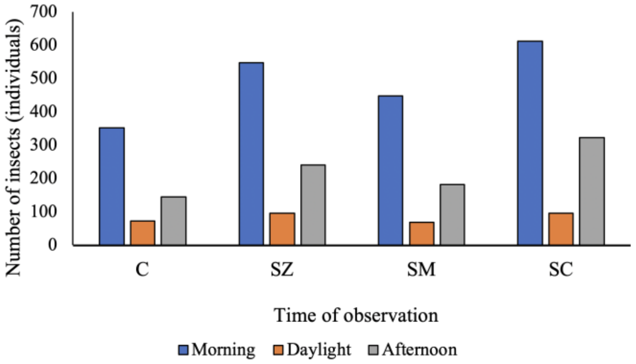

Figure 4. The abundance of insect pollinators at different periods. C: Control; SZ: Strawberry+common zinnia; SM: Strawberry+marigold; SC: Strawberry+cosmos

Table 3. Indices of diversity, evenness, and dominance of insect pollinators

Treatments	Diversity index (H')		Evenness index (E)		Dominance index (D)	
	Value	Category	Value	Category	Value	Category
C	1.825	Medium	0.336	Low	0.816	High
SZ	2.125	Medium	0.296	Low	0.857	High
SM	1.912	Medium	0.304	Low	0.834	High
SC	2.189	Medium	0.317	Low	0.864	High

Note: C: Strawberry/control; SZ: Strawberry+common zinnia; SM: Strawberry+marigold; SC: Strawberry+cosmos

Table 4. Strawberry yield data in each treatment with average and standard deviation

Subplot	Number of pollinators	Diameter of fruit	Length of fruit	Weight of fruit	Number of fruits
C-A	104	2.1	2.5	6.21	421
C-B	121	2.9	2.6	8.51	412
C-C	121	3.5	2.5	9.76	394
C-D	110	2.8	2.6	7.87	403
C-E	115	2.5	3.0	8.06	368
SZ-A	209	3.6	3.2	14.23	400
SZ-B	189	3.4	3.7	12.76	385
SZ-C	158	2.7	3.0	11.26	460
SZ-D	154	2.7	3.4	11.15	433
SZ-E	176	3.5	3.2	12.45	516
SM-A	151	2.7	3.6	11.34	391
SM-B	144	3.9	2.8	11.02	444
SM-C	139	3	3	10.76	317
SM-D	118	2.7	3	9.62	459
SM-E	148	2.5	3.5	11.12	476
SC-A	227	3.4	4.2	15.16	480
SC-B	201	3.8	3.5	13.98	409
SC-C	218	3.6	3.4	14.75	387
SC-D	188	2.9	3.9	13.04	493
SC-E	198	3.4	3.2	13.82	404

Note: The letters A, B, C, D, and E after the subplot code indicate the replication

The abundance of insect pollinators was analyzed by correlating it with the percentage of perfectly formed strawberry fruit due to complete pollination. The analysis showed a very strong positive relationship with r: 0.93. The results further prove that the abundance of pollinators in strawberry farming greatly improves the quality and quantity of strawberry fruit, which will eventually be more economically profitable. This result is in line with the research by Deepika et al. (2018) that incomplete pollination by pollinators on strawberry flowers can cause fruit malformation in 48.6% to 52.9% of all fruit production. Wietzke et al. (2018) reported strawberry fruit malformation can reach 92% when there are no pollinators in strawberry fields.

Strawberry quality is determined based on weight and fully formed fruit (not malformed). Based on the average fruit weight and the percentage of fully formed fruit, refugia flowers enhance fruit quality (Table 5) where such treatments yielded fully formed fruit with an average weight of more than 11 g. On the other hand, planting strawberries without refugia flowers produced fruits with an average weight of 8.08 g and only 58.44% of fully formed fruit with quality of category C (Figure 5). This result agrees with Masyitah (2018) that strawberries pollinated by various insect pollinators had a heavier fruit weight with a low proportion of malformed fruit, resulting in fruit quality of A (Figure 5).

The findings of this study concluded that refugia flowers can enhance the diversity and abundance of insect pollinators in strawberry farming. This condition eventually improves fruit production and quality. Adding cosmos flowers as refugia plants was the most effective in attracting strawberry pollinators, especially honey bees *A. cerana* and *A. dorsata*. Combining strawberry plants with cosmos (SC) also produced the highest fruit yield with the greatest weight and percentage of fully formed fruit.

Table 4. Fruit production, average weight, and percentage of perfect fruits in each treatment

Treatments	Number of fruits produced	Number of fully formed fruits	Weight of fruits (gr)	Percentage of fully formed fruits (%)
Control	1998	1167	8.08	58.41
Strawberry+common zinnia	2194	1775	12.37	80.90
Strawberry+marigold	2087	1497	11.05	71.73
Strawberry+cosmos	2173	1859	14.15	85.55

Figure 4. Malformed strawberry fruits caused by incomplete pollination

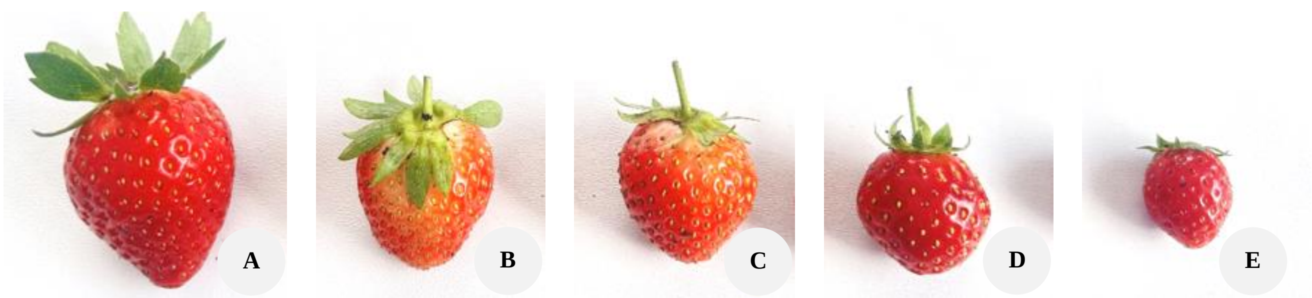

Figure 5. Quality of strawberry fruit based on weight and size: A. X>11 g, D: 3.4 cm, P: 4 cm; B. 9<X≤11, D: 3.2 cm, P: 3.3 cm; C. 6.5<X≤9, D: 2.5 cm, P: 3 cm; D. 3<X≤6.5, D: 2 cm, P: 2.9 cm; E. X≤3, D: 1.5 cm, P: 2 cm. X: Fruit weight; D: Diameter; P: Length

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