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RESEARCH ARTICLE - BEES

Linking bird resistant and susceptible sunflower traits with pollinator's fauna and seed production

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Abstract

Sunflower (*Helianthus annus* L.) is a highly cross-pollinated crop dependent on insect pollinators to provide a good quality edible oil worldwide. Different sunflower hybrids vary in terms of dependence on insect pollinators. Previously few studies have been conducted regarding the role of insect pollinators in hybrid sunflower seed production in Pakistan. Therefore, the current study was planned to explore the abundance and diversity along with foraging behavior (visitation rate and stay time) of native insect pollinators as well as to study the effect of different pollination treatments (free insect visits vs. no insect visits) on the reproductive success of different hybrids of sunflower. Two sunflower hybrids were grown at the research farm of MNS University of Agriculture, Multan, under the Randomized Complete Block Design (RCBD). In our study, the pollinator community consisted of honeybees (*Apis dorsata, A. mellifera,* and *A. florea*), solitary bees (*Pseudapis* sp.*, Megachilidae* sp. and *Xylocopa* sp.), and syrphid flies (*Eristalinus aeneus* and *E. megacephalus*). Furthermore, the relative abundance of pollinators was high in the H4 (bird resitant) having a flat head with 45º head angle from the stem hybrid, while the least abundance was observed in H3 (bird susceptible 180º head angle from stem). H4 proved to be a better hybrid among the hybrids regarding the number of seeds and seed weight. Both bees, i.e., solitary bees and honeybees, are crucial for pollinating sunflower. Comparative results of free insect visits and no insect visit treatments showed that the maximum number of seed weight, number of seeds, and seed diameter was observed in free insect visits compared to no insect visit treatment. Therefore, conserving the diversity of the native insect pollinators will lead to a higher yield of sunflower hybrids and other cross-pollinated crops.

Introduction

Sunflower (*Helianthus annuus* L.: Asteraceae) represents the 3rd source of raw matter in the world, contributing with more than 13% of the total edible oil production (Ramulu et al., 2011). Likewise, it contributes 16% in domestic edible oil production in Pakistan. Therefore, Pakistan still has a deficit in edible oil production and imports 1.98MT of edible crude oil by spending about 45 billion rupees (Khan et al., 2017; Ali et al., 2008).

In ecosystems, pollination plays a vital role in crop production. Nearly 84% world's crops are pollinated by insects (Bareke & Addi, 2019). Insect pollinators are the major contributors to about 5% of global food production (Carper et al., 2016). According to an estimate, the role of pollinators in the worldwide economy is USD 577 billion (Potts et al., 2016), and it contributes 1.59 billion dollars to the economy of Pakistan (Ahmad & Aziz, 2017).

Sunflower is an allogamous plant that requires insects in the flowering stage, particularly honeybees, to increase seed production (Chambo et al., 2011). Floral cues, shape, size, flowering timing, odor, color, and arrangement are vital in helping to attract pollinators to plants because flowers provide nectar and pollen (Nakata et al., 2021; Chittka & Walker 2006: Berjano et al., 2009). Research shows that sunflower has olfactory cues, including floral volatiles and a quantity of nectar and pollen that attracts bees to the flowers (Painkra & Kumaranag, 2019; Mallinger & Prasifka, 2017).

Some investigations also described that insect pollinators, especially the bees (wild and managed), increased seed yield by 18-65% on average in open pollination (where insects visited) (Degrandi-Hoffman & Chambers, 2006; Tamburini, Lami, & Marini, 2017; Stein et al., 2017). Previously, some studies have reported managed honeybees (*A. mellifera*) as the most abundant and effective pollinators due to their contribution towards seed weight, filled seed per head, and seed yield per head of hybrid sunflower (Rajasri et al., 2012; Abrol, 2012; Perrot et al., 2019). However, some other studies have reported solitary bees as more effective pollinators than honeybees (Mallinger et al., 2019). But some studies showed that honeybees' effectiveness increased with the presence of wild bees. In the absence of wild bees, nearly three seeds were produced by the single visit of honeybees, while in the presence, pollination efficiency increased up to 15 seeds on an average (Greenleaf & Kremen, 2006).

Studies have revealed that the flower angle affects pollination and increases pollination in zoophilous plants (Haverkamp et al., 2019). Some studies showed that plants with horizontal oriented flowers head enhance insect pollination compared to upward oriented flowers (Nakata et al., 2021; Wang et al., 2014; Ushimaru et al., 2009). At the same time, the upward orientation enhances the nocturnal (hawkmoth) pollination (Campbell et al., 2016).

Bird predation is a major problem in sunflower crop, resulting in seed loss. Breeders have developed such bird resistant sunflower hybrids with special characteristics (convex and flat shape, distance between head and stem that is higher than 15 cm, and horizontally oriented head), which makes the sunflower unfavorable for birds (Yasumoto et al., 2012, Khaleghizadeh, 2011, Prakash et al., 2010, Tarimo, 2000). On the contrary, bird susceptible cultivars have concave or flat shape capitula, the distance between the head and stem lesser than 15 cm, and vertically oriented heads as characteristics that enhance bird predation (Hladni et al., 2017; Khaleghizadeh et al., 2009; Parfitt, 1984). Previously, seed loss due to bird damage in susceptible sunflowers was 25-70% higher than in bird-resistant cultivars (Tomaz et al., 2019).

To the best of our knowledge, no work has been reported on bird resistant and bird susceptible varieties response to insect pollinators. Keeping in view this gap in research, our study aimed to test the following hypothesis: a) pollinator visiting increases sunflower reproductive success, leading to higher number of seeds and seed weight per head; b) sunflower plant architecture traits in susceptible and resistant bird predation hybrids affect: b1) insect pollinator abundance; b2) foraging behavior (visitation rate and stay time).

Materials and methods

Study site

The studies were carried out at the research farm of MNS University of Agriculture, Multan. Two sunflower hybrids (bird resistance and bird susceptible) were grown on an area of 0.25 acres in February for growing seasons (2021). The sunflower was sown on 0.762-meter ridges. The experimental plot and sowing of sunflower were homogeneously designed to avoid any variation that could affect the analytical comparisons of the experiment. Berseem (*Trifolium alexandrinum*: Leguminoceae) surrounded the crop in the South, maize (*Zea mays*: Poaceae) in the North, and quinoa (*Chenopodium quinoa*: Amaranthaceae) in the West; moreover, perennial trees including shisham (*Dalbergia sissoo*: Fabaceae), kikar (*Acacia karoo*: Fabaceae) and mulberry (*Morus alba*: Moraceae) were also present near sunflower fields.

Cultivars	Abbreviated	Cross
Bird resistant cultivar	H4	BR.11 x BR.82
Bird susceptible cultivar	H3	BS.16 X R36

The climatic condition of the study area was sub-tropical with cold winters and hot summers; the daily mean minimum and maximum temperatures ranged from 8 to 12 °C and 38 to 50 °C, respectively, and the mean monthly rainfall of summer was ca. 18 mm. The sunflower hybrids were grown in RCBD experimental design (Randomized Complete Block Design). There were two treatments, and each treatment was replicated three times. Plant to plant distance was 0.22 m, replication to replication distance was 3.04 m, and plot to plot distance was 1.52 m was maintained.

Plant characteristics

Plant characteristics data, i.e., head shape, head angle, plant height, chaff length, the distance of the head from the stem, and head positioning, were recorded according to Khaleghizadeh (2011).

Abundance and Diversity

The data of the abundance and diversity of insect pollinators in different hybrids of sunflower was recorded during clear sunshine days when the pollinators are fully active. The total number of sunflowers in each plot was 60, which was thrice replicated. For abundance, when 10% flowering started, ten sunflower heads were selected for each hybrid during each data census. Each sunflower head was observed for one minute to record the different abundance and diversity of varying insect pollinators (Jadhav & Prasad, 2011). Insect specimens were preserved for later taxonomic identification.

Diversity indices

Diversity indices are considered as those mathematical measures which refers to the diversity of species with respect to species richness (no. of species recorded from a particular area) and abundance (total number of individuals belonging to given species) from a community (Purvis and Hector, 2000; Schleuter et al., 2010). In this research, diversity indices are calculated with respect to type of impressions explored from the insect pollinator's species. Diversity indices were calculated individually from the bird resistant and bird susceptible cultivars. Species richness and species evenness are calculated with the help of Shannon diversity index (H), Shannon evenness measure (E_H), Simpson's diversity index (D), and Simpson's measure of evenness (E_D). Shannon diversity index (H) analyze the data through statistical information by keeping in view of diversity principle and is used globally to calculate the ecological diversity of species (Shannon, 1948). Simpson's diversity index (D) is a measure of dominance that includes the most abundant species recorded from a sample and least sensitive compared to species richness (Magurran, 2013). The formula for species richness and species evenness is given below:

$H = - \Sigma S\ i{=}1\ pilnpi$
$EH = H/lnS$
$D = 1/\Sigma s\ i{=}1\ pi^2$
$E_D = D \times 1/S$

Where H = impression-based Shannon diversity index,
EH = Impression-based Shannon evenness measure,
S = richness of impression
Types, pi = proportion of individual impressions that are of the Impression type,
ln = natural logarithm,
D = impression-based Simpson
Diversity index,
ED = impression-based Simpson evenness measure.

Foraging behavior

Foraging behavior (stay time, visitation rate) of abundant insect pollinators in different hybrids of sunflower was recorded. For visitation rate and stay time, five sunflower heads were selected for each plot, and a total of thirty values were taken of five species of pollinators. Visitation rate was observed in terms of the number of sunflower heads visited by a single pollinator in one minute per plot, while stay time was recorded in seconds spent by a pollinator on a single sunflower head (Mehmood et al., 2018).

Pollination treatments

Two pollination treatments, i.e., free insect visits (open pollination) and no insect visits (caged pollination), were used to compare insect pollinators' effectiveness in the reproductive success of sunflower. At the bud stage, before opening any floret, 10 sunflower heads per plot and 30 per treatment were selected and tagged randomly as open heads accessible to visitors (open pollination). At the same time, nine heads per plot and 27 seven heads per treatment were caged randomly by white nylon fine mesh bags (1mm mesh width) to exclude insect visitors, allowing only self-pollination (caged pollination). After harvesting, reproductive success parameters, i.e., head diameter, head weight, number of seeds per flower head, and seed weight per flower head, were compared between open and cage pollinated plants.

Reproductive Success

Following parameters of reproductive success were recorded, i.e., head diameter, the number of seeds per head, and seed weight per head (Tamburini et al., 2016). Thirty heads (20 from open and 10 from caged) of sunflower were harvested from each sunflower hybrid and measured diameter in centimeters. After the measurement of head diameter, the number of the seed of each harvested head were counted manually. The weight of seeds was measured with digital weight balance after the sanitation of seeds per head. For this purpose, we harvested thirty sunflower heads from each treatment.

Data analyses

The data were analyzed using Statistical Analysis System (SAS) software (SAS Institute, 2013). The Shannon and Simpson diversity indices and evenness data were subjected to t-test using PROC TTEST in SAS ($\alpha = 0.05$).

The pollinator visitation time and the stay time of pollinators on two cultivars were square root transformed and tested using t-test (PROC TTEST) in SAS ($\alpha = 0.05$). The difference among different species' visitation and stay time was tested using PROC GLIMMIX. The tukey's test was used to see differences among the mean values.

The data for head weight, head diameter, seed weight and numbers of seeds per head were subjected to PROC UNIVARIATE to check the normality. The data were then subjected to square root transformation to achieve the normality. The transformed data were subjected to PROC TTEST ($\alpha = 0.05$). The analyses were preformed individually for open and caged condition, followed by the combined (open + caged) plant parameters.

Results

Plant Characteristics

Both sunflower hybrid H4 and H3 have flat heads. According to table 01 Head angle of H4 was 90º while H3 had 180º while H4 had head positioning (8), and H3 had head positioning (9). Plant height, chaff length, distance of the head from the stem was observed higher in the H4 sunflower hybrid followed by the H3 sunflower hybrid (Table 1).

Table 1. Plant Characteristics of bird resistant and susceptible in sunflower cultivars.

Sunflower Hybrids	H4	H3
Head Shape	Flat	Flat
Head Angle	45°	180°
Chaff Length (Inch's)	17.48	14.4
Distance of head from the stem (cm)	5.2	5
Head Positioning	8	9
Plant Height	159.41	148.7

Abundance and diversity

During the flowering season of sunflower, six bee species (three solitary bees and three honeybees) belong to Hymenoptera, two syrphid flies belonging to Diptera and three lepidopterans species were observed. The overall abundance of pollinators was high in Bird resistant H4 (65%) hybrid as compared to Bird susceptible H3 (35%) cultivars (Table 2). Honeybees include *Apis dorsata, A. mellifera, A. florea* while in solitary bees *Xylocopa sp*., *Pseudapis sp* and *Megachilidae* sp, in syrphid flies include *Eristalinus aeneus* and *E. megacephalus* and in lepidopteran include *Pseudaletia unipuncta*, *Utethesia cardui* and *Pieridae rapae*. *A. mellifera* was the most abundant among all pollinators, followed by *A. dorsata,* while *Pseudaletia unipuncta* was the least abundant (Table 2).

Diversity indices

According to the outcomes of this research, in June and September 2019, values recorded for Shannon's and Simpson's diversity and evenness indices in bird resistant and bird susceptible cultivars were not statistically different with respect to the impression types investigated on diversity of insect pollinators. Greater values were seen for species evenness from bird resistant as compared to that of bird susceptible (Table 3).

Table 2. Abundance and diversity of pollinators in bird resistant and susceptible sunflower cultivars.

Order	Family	Genus/Species	H3	H4
Hymenoptera	Apidae	*Apis dorsata*	24	67
		Apis mellifera	94	149
		Apis florea	30	52
		Xylocopa sp.	4	17
	Halectidae	*Pseudapis* sp	22	37
	Megachilidae	*Megachilidae* sp.	10	22
Diptera	Syrphidae	*Eristalinus megacephalus*	19	47
		Eristalinus aeneus	32	41
Lepidoptera	Noctuidae	*Pseudaletia unipuncta*	2	4
	Nymphalidae	*Utethesia cardui*	3	5
	Pieridae	*Pieris rapae*	7	16
	Total		247 (35%)	457 (65%)

Foraging behavior (Visitation rate and stay time)

In this experiment, no significant difference was found for the visitation rate of the different pollinator species on the two hybrids. However, on H4 hybrid significantly higher number of *A. dorsata* and *Psedapis sp.* visited the flowers as compared to *A. florea* and *A. mellifera*, while no significant differences among different species were found on H3 hybrid (Table 4). The stay time of *A. mellifera, A. florea,* and *E. aeneus* was not significantly different in both cultivars while *A. dorsata* presented a significantly different stay time in H4 hybrid than in H3. *A. mellifera* had the highest stay time fallowed by *Pseudapis* sp. while the least stay time was observed in *A. florea* among all pollinators (Table 4).

Table 3. Means (± SE) of Shannon diversity index (H), Shannon's equitability (EH), Simpson's diversity index (D), and Simpson's equitability (ED) for pollinator diversity recorded on sunflower cultivars.

Variety	H	E_H	D	E_D
H3	1.553 ± 0.201	0.758 ± 0.100	4.235 ± 0.567	0.610 ± 0.080
H4	1.759 ± 0.137	0.833 ± 0.031	5.106 ± 0.545	0.565 ± 0.057
***t*, df**	-0.92, 18	-0.92, 18	-1.06, 18	-0.01,18
P	0.371	0.3714	0.304	0.989

Table 4. Mean (± SE) number of pollinators visiting and the pollinator stay time on bird-susceptible and bird-tolerant cultivars.

	Apis florea	*Apis dorsata*	*Apis mellifera*	*Pseudapis* sp.	*Eristalinus aeneus*	*F*, df	*P*
Visitation rate							
H3	0.52 ± 0.26	2.83 ± 0.49	1.93 ± 0.21	2.533 ± 0.40	2.36 ± 0.35	1.3, 4, 116	0.272
H4	1.86 ± 0.19b	3.10 ± 0.56a	1.67 ± 0.20b	2.26 ± 0.28a	2.03 ± 0.21ba	4.2, 4, 116	0.004
***t*, df**	1.65, 58	-0.27, 58	1.00, 58	0.47, 58	0.64, 58		
P	0.10	0.79	0.32	0.64	0.52		
Stay time							
H3	50.45 ± 6.49c	48.70 ± 6.59Bc	67.71 ± 7.14b	74.03 ± 9.19a	69.82 ± 8.79 ba	65.5, 4, 116	<.001
H4	67.19 ± 7.45c	99.44 ± 13.52Aa	92.91 ± 10.59a	55.47 ± 3.54d	84.14 ± 7.56b	122.5, 4, 116	<.001
***t*, df**	-1.84, 58	-3.11, 58	-1.90, 58	1.72, 58	-1.48, 58		
P	0.07	0.00	0.06	0.09	0.14		

Reproductive success in sunflower hybrids

The reproductive success in both sunflower hybrids H4 and H3 in terms of head diameter and head weight have no significant difference while seed weight and the number of seeds have significant difference in all conditions i.e. open, caged and both open + caged (Table 5). In open pollination, seed weight in H4 cultivar was higher (16%) than in H3 cultivar. The number of seeds per head in H4 cultivar was higher (46%) than in H3 cultivar. In open pollination, plants reached the maximum head diameter, i.e., 27% greater in H4 and 16% in H3 in comparison to caged heads (Table 5).

Table 5. Mean (± SE) head diameter, head weight, seed weight, and number of seeds recorded for two sunflower cultivars under the open, caged and combined (open + caged) conditions.

Condition	**Variety**	**Head diameter (cm)**	**Head weight (g)**	**Seed weight (g)**	**No. of seeds**
Open	H3	17.91 ± 0.38b	326.02 ± 22.43	70.32 ± 4.25	663.20 ± 41.41b
	H4	19.16 ± 0.38a	337.04 ± 14.60	86.11 ± 4.15a	1108.57 ± 46.21a
t, df		-2.30, 58	-0.66, 58	-2.57, 58	-7.07, 58
P		0.02	0.51	0.012	<.001
Caged	H3	18.07 ± 0.40a	190.05 ± 16.67	30.061 ± 1.06b	341.66 ± 14.95b
	H4	16.44 ± 0.38b	192.35 ± 12.71	39.96 ± 39.96a	524.11 ± 26.63a
t, df		2.95, 52	-0.26,52	-4.72, 52	-6.19, 52
P		0.04	0.79	<.001	<.001
Open + Caged	H3	17.98 ± 0.27	261.61 ± 16.75	51.25 ± 3.52b	510.89 ± 31.25b
	H4	17.87 0.32	268.50 ± 13.67	64.25 ± 3.86a	831.71 ± 47.52a
t, df		0.32, 112	-0.53, 112	-2.57, 112	-5.73, 112
P		0.7	0.59	0.01	<.001

Discussion

The current study showed that the overall abundance of pollinators was higher in bird resistance H4 cultivar compared to bird susceptible H3 cultivar. Among all the pollinator species visiting sunflower cultivars, *A. mellifera* was the most abundant pollinator followed by *A. dorsata* and *A. florea.* No significant differences were found in the visitation rate of the different pollinator species on the two hybrids. *A. dorsata* stayed for significantly greater time on H4 cultivar than on H3. Significant differences in seed weight and number of seeds were observed in all pollination treatments, i.e., open, caged and combined (open + caged) conditions.

Apis spp. were found to be abundant species visiting the sunflower cultivars. Visitation of *A. mellifera* and *A. dorsata* as dominant pollinators, followed by the solitary bee *Trigona iridipennis*, has been recorded on sunflower hybrids (Jadhav et al., 2011; Rajasri et al., 2012). In another study, *A. mellifera* was the most frequent visitor in different sunflower hybrids (Oz et al., 2009; Hoffman & Chambers, 2006; Kasina et al., 2007; Mallinger & Prasifka, 2017). For the oilseed crops honeybees were found to be abundant in mustard (*Brassica juncea*: Brassicaceae) (Shakeel et al., 2019; Delaplane et al., 2013; Parbat, 2019), canola *Brassica napus*: Brassicaceae) (Akhtar et al., 2018; Amro, 2021), and sesame (*Sesamum indicum*: Pedaliaceae (Das et al., 2019; Pashte et al., 2013). Previous studies from Pakistan indicated *A. mellifera* as an abundant and efficient pollinator of sunflower (Akhtar et al., 2018; Ali et al., 2015). The abundance of the *Apis* spp. indicates pollinator preference to forage on sunflower.

Pollinator species showed significant differences for visitation rate on bird resistant sunflower cultivar (H4). Visitation rate is an important parameter for evaluating the efficiency of insect pollinators, especially native species (Albano et al., 2009). *Apis mellifera* has been reported as a frequent visitor on sunflower crop in multiple studies (Estravis Barcala et al., 2019 & Nderitu et al., 2008). Similarly, wild bees like *Melissodes* spp. and *Andrena helianthus* have also a frequent visiting activity on the sunflower (Mallinger et al., 2019; Mallinger et al., 2015 and Greenleaf & Kremen, 2006). In another study, the frequency of *A. mellifera* ranged from 2.27 to 2.94 bees per sunflower head (Chambó et al., 2011). Overall, the visitation frequency could also be linked with the body size of bee species, in addition to other factors (Everaars et al., 2018).

The number and weight of seeds were higher in bird resistant cultivar (H4). Pollinator species, like *A. mellifera* have been reported to induce better seed setting in terms of number and weight of seeds (Jadhav et al., 2011; Rajasri et al., 2012; Martin et al., 2016), while in the case of pollinator restricted arenas, 18-25% less seed set was observed for sunflower hybrid (Hoffman & Chambers, 2006; Mallinger & Prasifka, 2017). So, the pollinator activity leads to a better seed health and seed set, as found in the current study.

To conclude, the cultivar doesn't change the structure of the flower-visiting guilds (richness and abundance) but the change in the total abundance might lead to a higher oilseed yield. The selection of sunflower cultivars is an important pollination factor of sunflower in all aspects, i.e. abundance and diversity and foraging behavior (visitation rate, stay time) of insect pollination which may ultimately increase or decrease the sunflower yield. The sunflower bird resistant cultivar has been found leading to an increase of bee abundance with a coupled effect in reproductive success and yield increase. Further research should focus on screening multiple sunflower cultivars for pollinator activity with reference to the flower head angle.

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Authors' Contribution

SI: conceptualization, data curation, investigation and writing: original draft.
MA: conceptualization, funding acquisition, planning experiment, investigation, supervision.
FZAK: formal analysis, writing: original draft.
NI: writing: review & editing.
FN: writing: review & editing.

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