



Pollination Deficit in Apple Orchards at Murree, Pakistan

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ABSTRACT

A study was conducted in twenty different managed and unmanaged apple orchards of Murree for determining relationship of different pollinator groups with crop yield. Apple is dependent on insect pollinators to set fruit. Farmers in Pakistan are generally not aware of pollination needs of apple. Results depicted a high population decline of Syrphids and Non-Apis bees. Syrphids were recorded as 12.65 to 13.85 per 250 flowers in 2013 but decreased in year 2014 by 7.82 to 7.88 per 250 flowers and Non-Apis bees was recorded as 6.15 to 7.59 per 250 flowers in 2013 which also decreased and recorded as 5.35 to 5.70 per 250 flowers in year 2014. The results showed the trends of increase in population of *Apis mellifera* and *Apis cerana* between 8.22 to 11.5 per 250 flowers in 2013 as compared to 1.12 to 13.92 per 250 flowers in 2014, whereas population of indigenous *Apis cerana* varied between 3.80 to 5.44 h per 250 flowers in 2013 as compared to year 2014 where it was recorded as 4.34 to 5.56 honey bees per 250 flowers. Apple fruits yield per tree ranged between 214.56 to 218.64 in 2013 and 2014 respectively. Average fruit weight varied between 124.84 to 127.34 g, average number of seeds varied between 7.95 to 7.73 seeds per fruit and yield per tree was recorded between 28.92 to 31.65 kg per tree. Number of Apple fruits per tree ranged between 145.72 to 164.58 in 2013 and 2014, respectively. Average fruit weight varied between 103.66 to 112.13 g and average number of seeds varied between 7.73 to 7.95 seeds per fruit and yield per tree was recorded between 17.34 to 21.78 kg per tree. Eighteen different species were recorded under 16 genus and 07 families. *Ceratina hieroglyphica*, *Halictus subauratus*, *Osmia caerulescens* were reported first time from Pakistan.

Article Information

Received 11 May 2016

Revised 23 September 2016

Accepted 21 January 2017

Available online 02 May 2017

Authors' Contribution

RM conceived and designed the study and also wrote the article. WA and GS collected the data. MKR analyzed the data. AS identified pollinators.

Key words

Apple, Managed apple orchards, Unmanaged apple orchards, Pollinators, Syrphids, *Apis mellifera*, *Apis cerana*.

INTRODUCTION

Nearly 80% of total apple production of Pakistan is contributed by Balochistan and is mainly produced in Kalat, Killa Saifullah, Loralai, Mastung, Pishin, Quetta and Ziarat districts. The area under this fruit has explicitly increased more than five times during the period from 1980-81 to 1996-97 (Anonymous, 1999). At present, it is unfortunate to observe that yield of apple is far below the potential. Gap between the actual and potential yields of apple orchards could be reduced by modern agronomic practices, higher inputs as well as proper pollination management. Insects play a vital role in pollination of various crops particularly in apple orchards. Indiscriminate use of pesticides in modern agriculture has disturbed the ecological inter-relationship by massive killing of farmer friendly insects along with detrimental insects (Verma and Partap, 1993). In Pakistan, one symptom of the shortage of friendly insects is alternate years of fruit bearing in most of apple orchards in Balochistan most probably due to lack

of pollinating agents and another indication is overtime decline in fruit yields in North West Frontier Province (NWFP) of Pakistan (Partap and Partap, 1997).

Pollination deficit means 'quantitative or qualitative inadequate pollen receipt that limits the sexual reproductive output of plants' (Wilcock and Neiland, 2002). The inadequate pollen receipt may be quantitative/qualitative due to lacking value of the pollen grains deposited, or inadequate with respect to timing, that is occurring outside the period of effective pollination based on stigmatic receptivity and ovule senescence. Pollination is a necessary step in production of most fruits and many vegetables. This process has been observed as a free ecosystem service donated by the nature. However, pollination done by insects is much valuable in case of both cultivated crops and in uncultivated areas (Irshad and Stephen, 2012). More seeds were produced in fruits which pollinated by bumblebee as compared to manually and self-pollinated fruits (Munir *et al.*, 2015). An estimated 35% of the global production of plant-based food comes from crops that benefit from animal pollination (Klein *et al.*, 2007). Bees are by far the most important pollinators in agricultural settings and contribute between \$5.7 to \$19 billion per year to the United State economy (Morse

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0030-9923/2017/0003-0897 \$ 9.00/0

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and Calderone 2000) and \$217 billion per year globally (Gallai *et al.*, 2009). Native bees play an important, but underappreciated, role in crop pollination. The economic contribution of our native bees to agricultural pollination is almost certainly high, but has rarely been quantified (Ricketts, 2004).

Pollination is the mechanical transfer of pollen from the male to female portion of the same or different flower. This is fastening phenomenon for the development of fruit and seeds in flowering plants. Pollination is as important as other inputs (Seed, Fertilizer, and Irrigation) for the better production and is rather inevitable for fruit production (Khan and Chaudhry, 1988). Approximately 80 percent of all flowering plant species are specialized for pollination by animals, mostly insects (Ascher and Rasmussen, 2010). The available records show that many wild pollinators have declined radically in recent decades. These declines are almost certainly the result of escalation of farming practices during the second half of the 20th century (Williams, 1986; Buchmann and Nabhan, 1996; Westrich, 1996; Westrich *et al.*, 1998; Osborne and Corbet, 1994). Notable changes include the loss of unimproved flower-rich grasslands, loss of hedgerows (removing food sources for insects) and the widespread use of insecticides and herbicides.

Pollinators' fauna of what presently called as Pakistan was first studied by Bingham (1897) in his monograph of the bee fauna of British India. Later on most of the research work was carried out to assess the monetary benefits derived by the pollination by honey bees. Some work on pollinators of oil seed crop and fruit crops was also conducted. Most recently Irshad and Stephen (2010) presented the bibliography of the pollinator's fauna of Pakistan. Ascher and Rasmussen (2010) gave the Bee Species list for Pakistan.

Apple is an important fruit crop of Pakistan. Average annual production of apple fluctuates between 0.3-0.4 million tons during last five years. The insects as pollinator being important production factor can play a significant role. The present study was undertaken to see if pollination in apple is affected by the type and variety of pollinators and their effect on crop yield.

MATERIALS AND METHODS

Apple orchards selection

Twenty orchards were selected at the nucleus STEP Site Murree during the each year 2013 and 2014. The orchards were split into two major groups *i.e.* managed and unmanaged. Under this procedure, 10 orchards were selected for keeping a honey bee (*A. mellifera*) colony during the experiment interval whereas the remaining 10 were kept without a honey bee (*A. mellifera*) colony.

The treatment combination of each set is Study field without honey bee hive and with honey bee hive. Each study field was separated from other by a distance at least equal to 2km and if possible greater than the maximum modal foraging distance of managed pollinators. In the year 2013, the experiment started on 5th April, while in 2014 it started on 15th April, 2014 on the onset of 10% flowering on Apple plants at Murree. Each study site was visited four times (every 3rd/ 4th day or as the weather permits) during the blossom period. Data were collected by scanning of flowers, collection of insects by pan trap method and collection of pollinators by net sweeping. These measurements were recorded in the experimental sites under good weather conditions for foraging bees: temperature $\geq 15^{\circ}\text{C}$, low wind, no rain, and dry vegetation (Westphal *et al.*, 2008).

Pan trap collection

Twenty four bowls were placed and each bowl was at the distance of 2 meters in transecting position. The bowls of three different colors were used *i.e.* blue, white and yellow. The bowls contained a solution of detergent and water. The specimens were collected and handed over to the National Insect Museum (NIM, NARC) for sorting, pinning and identification purposes.

Scan sampling

The method of scan sampling was performed during the apple blooming period (starting from 10% flowering and onwards) to estimate the density of insect pollinators in orchards under different treatments. The data was recorded on the sites during the main flowering season *i.e.* April 2013 and 2014. In each orchard, four plots were selected and each plot consisted of 1 production tree. Almost 250 flowers were observed on each tree in estimated 15-20 min.

Net sweeping

The insects pollinators were caught during 8:00 am to 3:00 pm with aerial nets along six 25 m long and 2 m wide transects over 5 min each, for a total of 30 min per study field. The collected specimen sent to NIM, NARC, Islamabad for Identification

Yield of apple

All the 4 numbers of plots (1 apple tree each) were chosen for counting of fruits in each orchard. In the end, fruits of 4 trees were counted to make out the total production of apples from each orchard and the total fruit weight was done by the weighing balance. Fruit weight (g) and size (mm) of collected fruit samples were recorded with the help of electronic balance and Vernier calliper in the laboratory, respectively.

Table I.- Insect pollinator species in apple flower, with their total abundance counted by different methods.

Genus/Species	Abundance (Pan trapping) weekly Avg.				Abundance (Scan sampling/250 flowers) Avg./Week				Abundance (Net sweeping) one time sweeping			
	Managed		Unmanaged		Managed		Unmanaged		Managed		Unmanaged	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
<i>Apis</i> sp.	26	27	24	28	9.15	20.49	13.2	5.3	17	45	15	42
<i>Bombus</i> sp.	11	10	11	23	0.34	0.79	0.36	0.79	7	15	15	15
<i>Xylocopa</i> sp.	6	9	6	13	0.34	0.48	0.36	0.58	10	59	4	12
Non <i>Apis</i> /Solitary bees	358	343	358	388	7.59	5.7	6.1	5.3	43	89	58	87
Syrphids	450	479	450	425	13.9	7.88	12.7	7.82	457	472	435	439
Drone flies	155	178	155	210	1.5	0.87	0.98	0.65	138	153	107	93
Misc. dipteran flies	999	941	999	1048	1.3	0.56	2.3	1.3	206	290	185	200
<i>Lucilia</i> sp.	995	883	995	976	2.1	0.02	2.1	0.78	0	0	0	0
Wasp	15	31	15	31	1.4	0.53	1.4	0.54	2	63	4	8
Beetles	7	11	7	9	1.9	2.3	1.2	1.9	21	223	18	40
Butterflies	113	142	113	198	1.65	1.79	1.36	1.65	167	156	138	223

For further analysis of fruit, every fruit sample was marked *i.e.* no. of seeds per fruit, length and breadth of the fruit.

RESULTS AND DISCUSSION

Pollinators collected at Ghora Galli Muree presented in Table I showed that about 18 different species are recorded under 16 genera and 07 families. *Ceratina hieroglyphica*, *Halictus subauratus*, *Osmia caerulescens* are reported first time from Pakistan. The pollinator community of apple was composed of 26 bee (Hymenoptera) species, 18 true fly (Diptera) species, and 6 butterfly (Lepidoptera) species. A smaller proportion of these species was found during our systematic observations, *i.e.*, 6 bee species, 10 true fly species, and 1 butterfly species (Table I). Among the pollinators, *Apis mellifera*, *Apis florea*, *Apis cerana*, *Andrena* sp., *Ceratina hieroglyphica*, *C. binghami*, *Lysioglossum* sp., *Lasioglossum* sp., *Osmia caerulescens*, *Megachile rotundata*, *Halictus* sp., *H. smaragdulus* sp., *Xylocopa iridipennis*, *E. corolla*, *E. confrater*, *Episyrphus balteatus* which came under systematic observation whereas *Xylocopa* sp., *Anthophora* sp., *Nomia oxybeloides*, *Nomia* sp., and *Nomioides* sp. were rarely seen. Syrphidae was the dominant family with six species, followed by three species of Apidae, and a single species (*Andrena* sp.) was observed from the *Andrenidae*. *Apis dorsata* and *A. florea* (Apidae) were the most frequent floral visitors with totals of 492 and 73 individuals, respectively. Their average visitation frequency was also highest among all the observed bee species (Table II). The remainder of the

observed bee species was very low in abundance and hence their visitation frequencies were not recorded. The family Syrphidae was the most dominant among the true flies, comprising nine species. Six out of the nine syrphids were included in the floral visitor census (Table I), including the most abundant *Eristalinus aeneus* and *Ischiodon scutellaris*, with identical highest visitation frequencies. *Sphaerophoria bengalensis*, *Scava latimacullata*, and *Eristalinus arvarum* were occasional syrphids and were only recorded as part of the pollinator community. The remaining seven Diptera families included only one to three species each. The *Lucilia* sp. was the most dominant, having the highest abundance (883-995 individuals) and visitation frequency (Table II). Butterflies were more limited floral visitors both in abundance and diversity (number of species); only one (*Danaus chrysippus*) came under systematic observation and another five (*Junonia almana*, *Anaphais aurota*, *Eurema hecabe*, *Colotis vestalis* and *Pieris brassicae*) were rarely seen.

Table II.- Yield attributes the managed and unmanaged apple orchards, during 2013 and 2014.

Yield Attributes	Managed		Unmanaged	
	2013	2014	2013	2014
Avg. no. of fruits/tree	214.56	218.64	145.72	164.58
Avg. fruit weight (g)	124.84	127.34	103.66	112.13
Avg. no. of seeds/fruit	7.95	7.73	7.9	7.95
Avg. yield / tree (kg)	28.92	31.65	17.34	21.78

Co-efficient of correlation was calculated for all the observed variables. All the variables were found to be positively associated with one another. However, some variables had strong (correlation value close to 1) relationship. Pollinator's visitation has strong positive correlation with fruit quality and fruit weight. It can also be interpreted from the results that with increasing number of visitors per flower, fruit weight and fruit quality is enhanced. Similarly numbers of seeds per fruit were also strongly associated with the average number of visitors. It can also be concluded that *Apis mellifera*, *Apis florea*, *Apis cerana*, *Andrena* sp., *Ceratina hieroglyphica*, *C. binghami*, *Lysiglossum* sp., *Lasioglossum* sp., *Osmia caerulea*, *Megachile rotundata*, *Halictus* sp., *H. smaragdulus* sp., *Xylocopa iridipennis*, *E. corolla*, *E. confrater*, *Episyrphus balteatus* which increased the fruit weight and quality. Conclusively, it can be depicted safely that pollinators diversity and average number of visiting pollinators contributed to the fruit weight and fruit quality. There is a weak but highly significant relationship between fruit weight per flower and average number of insect visitors. It was just not one bee hive of *A. mellifera* which was placed at each study site but there was a wide variety of pollinators such as bumble bee, syrphid, *Apis* and others that make this relationship significant (Supplementary Tables I-IV). Diversity in insect pollinators, therefore, significantly enhanced the per flower increase in fruit weight in the farm.

Pollinators collected at Ghora Galli which showed that among bees maximum number were of *Ceratina hieroglyphica* (41) followed by *Osmia caerulea* (12). Thus *Ceratina hieroglyphica* seems to be more prevalent than other species. Among others, maximum numbers were of *Lucilia* sp. (41) commonly called blow flies followed by *Episyrphus balteatus* (syrphid flies). About 18 different species are recorded under 16 genus and 07 families. *Ceratina hieroglyphica*, *Halictus subauratus*, *Osmia caerulea* are reported first time from Pakistan. The results obtained by the Pan Trap Method showed that the managed orchards and the unmanaged orchards had no significant difference in the population trends whereas the yearly data depicted some variation in some groups of insects *i.e.* the no. of solitary bees, drone flies, misc. dipteran flies, misc. butterflies increased in the year 2014 than that of in 2013 (Supplementary Tables I-IV).

The comparison of scan sampling data for the year 2013 and 2014 depicted a high population declines in that of syrphids and non-*Apis* bees *i.e.* syrphids were recorded as 12.65 to 13.85 per 250 flowers in 2013 but decreased in the year 2014 by 7.82 to 7.88 syrphids per 250 flowers and non-*Apis* bees was recorded as 6.15 to 7.59 per 250 flowers in 2013 which also decreased and recorded as 5.35 to 5.70

per 250 flowers in the year 2014. The trends showed an increase in the population of *A. mellifera* and *A. cerana*, as the recorded nos. of *A. mellifera* varied between 8.22 to 11.5 honey bees per 250 flowers in 2013 as compared to 1.12 to 13.92 honeybees per 250 flowers in 2014 whereas the population of Indigenous honeybee *A. cerana* varied between 3.80 to 5.44 honey bees per 250 flowers in 2013 as compared to the year 2014 where it was recorded as 4.34 to 5.56 honey bees per 250 flowers (Table I).

Apples are self-incompatible; they must cross-pollinate to develop fruit. During the flowering each season; apple growers often utilize pollinators to carry pollen. Honey bees are most commonly used. Orchard mason bees are also used as supplemental pollinators in commercial orchards. Bumblebee queens are sometimes present in orchards, but not usually in enough quantity to be significant pollinators. Varieties are sometimes classified by the day of peak bloom in the average 30-day blossom period, with pollinizers selected from varieties within a 6-day overlap period.

Declines in pollinator populations around the globe, the recent loss of honey bees (*Apis mellifera* L.) to colony collapse disorder, and increased awareness of the lack of baseline data regarding pollinators other than honey bees for crops and other ecosystems has led to a surge in interest in non-*Apis* pollinators (Hackett *et al.*, 2010). Though we generally view the European (or western) honey bee as our most important pollinator, native and introduced non-*Apis* bees provide the majority of pollination services for many crops in the United States (Richards, 1993). In Virginia, most research on bee pollinators has focused on honey bees, bumble bees, *Bombus* spp. (Family Apidae), the orchard mason bee, *Osmia lignaria* Say (Family Megachilidae), and two species of squash bees, *Peponapis pruinosa* Say and *Xenoglossa strenua* Cresson (Family Apidae), although nearly 500 species of bees inhabit the state (Burley, 2007).

The honey bee (*Apis mellifera*) is usually regarded as the sole pollinator and is said to pollinate more than the one third of global crops directly or indirectly (Williams, 1995); however, its effectiveness is quite controversial (Westerkamp, 1991; Allen-Wardell *et al.*, 1998). Several authors have shown that wild bees were more effective pollinators than honey bees. Syrphid flies are also efficient pollinators of oilseed rape (Jauker and Wolters, 2008) and are characteristic of spring in Punjab Pakistan (Saeed *et al.*, 2008). Most of the bees primarily consumed nectar; pollen attached to their body is an extra reward. Pollinator plant interaction depends on the energy needs of the pollinators and the energy available from plants (Abrol, 1986). The proportion of pollen or nectar in flowers may change the behavior of an insect and, ultimately, pollination

effectiveness (Adegas and Couto, 1992), so a nectar feeder may change the extent of nectar robbing in which it participates. In canola flowers, the availability of nectar and pollen rewards decreases at 1200 h (Meyerhoff, 1954); this may explain why *A. florea* robbed nectar more in the afternoon than in the morning, because nectar robbing is a “minimal effort” way to find nectar. Muhammad *et al.* (2016) found that intercrop, diversity and average number of visiting pollinators contributed positively to the fruit weight and fruit quality. Pollinators collected from Multan belonged to seventeen different species under 15 genera and 07 families. *Ceratina binghami* apparently seems to be the dominant bee species followed by *Apis andreniformis* though no significant difference was observed. Among flies, *Episyrphus balteatus* seems to be dominant fly visiting mango orchards compared to other pollinators. Future studies should consider native pollinators conservation and management for their pollination effectiveness together with basic studies on their biology, for example nesting site locations and alternative nectar resources.

Yield attributes comparison

Managed orchards

In the managed group of Apple orchards, the number of apple fruits per tree ranged between 214.56 to 218.64 apples per tree during the year 2013 and 2014, respectively. Whereas the average fruit weight varied between 124.84 to 127.34 g and the average number of seeds varied between 7.95 to 7.73 seeds per fruit, and the yield per tree was recorded between 28.92 to 31.65 kg per tree.

Unmanaged orchards

On the other hand, unmanaged of Apple orchards, the number of apple fruits per tree ranged between 145.72 to 164.58 apples per tree during the year 2013 and 2014, respectively. Whereas, the average fruit weight varied between 103.66 to 112.13 g and the average number of seeds varied between 7.9 to 7.95 seeds per fruit, and the yield per tree was recorded between 17.34 to 21.78 kg per tree. The severe weather conditions like rains and hailstorm during flowering and fruiting seasons were the main cause for low fruiting in some orchards *i.e.* Osia, Dahla, Rawat. In the concluding results, the apple orchards with honey bee colony yielded maximum fruit, both in terms of quality and quantity (Table II).

In spite of the fact that inadequate studies exists in Pakistan on pollination deficit still the previous work shows that production of certain crops can be enhanced by putting some efforts on pollination activity in the field. There was considerable variation in levels of pollinator dependency which could be due to effects of site, varieties of crops grown and inputs, which we could not test

because of limitations of study design. Our results did not support many cases in literature: For example orchards having low levels of reliance on pollinators for crop production (Klein *et al.*, 2007) showed significant levels of pollinator dependency in our study described as having moderate levels of pollinator dependence. Therefore, more data from different sites are required to get a realistic picture of pollinator dependence of sub tropical crops. Diverse pollinator assemblages visiting flowers could be due to small scale farms having adequate shrubbery and weedy vegetation. Uncultivated areas and shrubbery around farms are an important refuge for insects and buffer the effects of insecticides. Our study also identifies syrphid flies as a possible major pollinator group. Bees, particularly honeybees have rightly been given the key role of pollinating crops worldwide (Potts *et al.*, 2010). The correlation matrix for each category of orchards is given in Supplementary Tables I-IV.

CONCLUSIONS

Number of honey bee *Apis mellifera* L. hives, their active duration and preference for a specific variety may serve as important factors to maintain their optimum activity. Present studies stress the use of honeybees hives as regular incorporation at the time of flowering of apple crop. Selection of high yielding cultivars can help to increase the economic returns when these foraging bees can result in 100% crop pollination. It is suggested to use honeybees as crop pollinators for better and improved apple crop under current cropping pattern of apple farming in Pakistan.. More foraging of honeybees in apple need to be adjusted on spatial flower distribution which needs to be considered to maximize their foraging and ultimately their crop yields.

ACKNOWLEDGEMENTS

This work was conducted under GEF/FAO Global pollination project on “Conservation and management of pollinators for agriculture through ecosystem approach”. The financial and technical help is highly appreciated and acknowledged.

Supplementary material

There is supplementary material associated with this article. Access the material online at: <http://dx.doi.org/10.17582/journal.pjz/2017.49.3.897.903>

Statement of conflict of interest

Authors have declared no conflict of interest.

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