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Bee diversity assemblage on pigeon pea, *Cajanus cajan* along habitat gradient

Gurpreet Singh Makkar[1] and Pardeep K. Chhuneja[2]*
[1]Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana–141 004, India
[2]Department of Entomology, Punjab Agricultural University, Ludhiana–141 004, India
*Corresponding Author E-mail: **pkchhuneja@pau.edu**

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Abstract

The regional bee diversity was investigated on pigeon pea in three agroclimatic zones of Punjab state in northern India. Of the total nine species recorded, population of Megachilids, in particular, was significantly higher in all the three zones. Appraisal of diversity metrics revealed highest bee community diversity in Sub-mountain Undulating zone, followed by Central Plain zone, while least diversity was noted in Western zone, which highlighted the effect of habitat on species richness and abundance. Diurnal variations were evident with the most diverse bee communities recorded at 11:00 hr, followed by at 14:00 and 08:00 hr, with the lowest community diversity at 17:00 hr. The relative abundance of bee species was highly in favour of non-*Apis* species than *Apis* species. The results obtained can be used in the application of risk management through planned plant protection measures, study of plant-pollinator interactions along with conservation and augmentation of bee species.

Key words

Agroclimatic zones, Bee pollinators, *Cajanus cajan*, Diurnal abundance, Species diversity

Introduction

Pollination is one of the most significant activity of bees (Hymenoptera: Apoidea), allowing sexual reproduction in the majority of food crops of the world. Bees are diverse taxa with estimated 20,000–30,000 species worldwide (Michener, 2007), and the most recent account includes 20,092 valid names (Ascher and Pickering, 2015), organized under seven families. Several studies have demonstrated the economic value of bee pollinators to the agricultural industry worldwide (Klein *et al*., 2007; Michener, 2007), however, a recent economic estimate suggested that honey bees and other pollinators taken together contribute to world agriculture an estimated worth of over €153 billion annually (Gallai *et al*., 2009).

Pigeon pea (*Cajanus cajan*) commonly known as Tur or arhar is an important annual food legume crop of semi-arid tropics for subsistence agriculture due to its drought and excessive moisture tolerance, high-protein (20–25%) grains, adequate supply of essential amino acids, especially lysine, quality fodder, fuel wood and its ability to enrich soil. In India, it is the second most important pulse crop after chickpea, cultivated on an area of 4.42 million ha with average productivity of 647 kg ha^{-1} (FAOSTAT, 2013). Though, the floral biology of pigeon pea favours self-pollination, yet considerable degree of natural cross-pollination occurs, ranging from 12.6 - 45.9 % in India (Onim, 1981; Saxena, 2006). Various *Apis* and non-*Apis* bee species visit pigeon pea blooms (Grewal *et al*., 1990; Ahmad and Srivastava 2002; Saxena and Kumar, 2010), effecting 10 - 65 % cross pollination (Rao and Suryanarayana, 1983; Daspute *et al.,* 2014) with improvement in yield and quality characteristics (Rashmi *et al*., 2010).

Despite some earlier reports, updated information on the species diversity (abundance, richness and evenness) associated with this crop, their diurnal variations and pattern of distribution in different agroclimatic zones of Punjab is completely lacking. Such studies are essential for initiating the habitat management and conservation programmes for the native pollinator fauna and application of risk assessment to pollination for simple considerations of pesticide usage and their registration in the light of their potential environmental and economic impacts.

In light of above, systematic investigations were planned with the following objectives : species richness and diversity of bee pollinators on pigeon pea in various agroclimatic zones of Punjab; diurnal abundance of bee species on *C. cajan* in relation to different times of the day. In addition, attempts were also made to generalise the ecological health of different zones by correlating the diversity of bee communities with the existing information on ecological conditions of these zones.

Materials and Methods

Bee pollinators' diversity was monitored in three major agroclimatic zones of Punjab state (northern India) viz., Central Plain zone (31°15'24" N and 75°46'47"E; 31°36'4" N and 75°39'50"E), Sub-mountain Undulating zone (31°20'32" N and 76°1'57"E; 31°19'55" N and 76°3'10"E) and Western zone (30°24'29"N and 74°31'32"E; 30°21'16"N and 74°27'19"E) (Anonymous, 2015) to include a wide range of habitats, cropping areas, ecological conditions, soil types and surrounding environment. At each collection site, the bee foragers on the crop were trapped four times a day through net sweeping. Ten sweeps were made at three-hour intervals *i.e.* 08:00 hr, 11:00 hr, 14:00 hr and 17:00 hr at each location, once a week for consecutive three weeks, at four randomly selected locations (replications) each of 5x5 m, in a plot of minimum of half acre size. The observations were recorded during peak flowering period of crop, spanning over the months of September-October for two years (2012 and 2013) to ensure collection of all pollen vectors associated with the crop.

The bees collected from each site, were sorted both location and time-wise in the Apiculture Laboratory, Department of Entomology, Punjab Agricultural University, Ludhiana for their subsequent identification, grouping and counting. The bee specimens were deposited at National Insect Museum, Department of Entomology, PAU, Ludhiana and NPC, ICAR, New Delhi (India).

Data on diurnal bee abundance were transformed using $\sqrt{n+1}$ transformation to fulfil the assumptions of Analysis of Variance (ANOVA) for count data. The treatment means, representing the number of pollinators per 30 net sweeps (mean of 12 observations, *i.e.* 3 weeks x 4 locations) were analysed through a factorial completely randomized design using ANOVA and were separated by least significant difference (LSD) at p=0.05 level (Gomez and Gomez, 1984). Twelve diversity indices comprising two species number indices [Margalef's Index, D(Mg) and Menhinick's Index, D(Mn)]; six proportional abundance indices [Brillouin Index, HB; Shannon-Weiner Index, H'; Inverse Simpson's Index, 1/D(S); McIntosh's U index; Inverse of Berger-Parker's Index, 1/d and Cuba's Index, DC]; two evenness indices [Shannon evenness, E of H' and McIntosh evenness, E(McIn)] and two dominance indices (Southwood dominance, D or 1-J and McIntosh's dominance index, D(McIn) were calculated according to Magurran (2004). Each set of indices was ranked and Kendall's coefficient of concordance (W), was calculated according to Siegel and Castellan (1988).

Results and Discussion

In all, nine species of bee pollinators belonging to two different families of Hymenoptera were recorded in three agroclimatic zones of Punjab, which revealed maximum representation of family Apidae with seven species viz., *Apis mellifera* Linnaeus, *Apis cerana* Fabricius, *Apis dorsata* Fabricius, *Apis florea* Fabricius, *Ceratina binghami* Cockerell, *Xylocopa fenestrata* Fabricius and *Xylocopa aestuans* (Linnaeus) followed by two species belonging to family Megachilidae viz., *Megachile lanata* Fabricius and *Megachile bicolor* Fabricius.

The Central Plain zone had highest bee species richness (S = 9) on *C. cajan* (Table 1). *M. lanata* was the most abundant species (16.0±1.1/ 30 net sweeps, RA = 22.8%) followed by *M. bicolor* (14.6±1.2/ 30 net sweeps, RA = 20.8%), whereas the mean abundance of *A. cerana* (0.9±0.3/ 30 net sweeps, RA = 1.3%) was significantly lowest among all. Collectively, seven species of Apidae (RA = 56.4%) outnumbered two species of Megachilidae (RA = 43.6%). Non-*Apis* bees recorded higher species richness and abundance (S = 5, RA = 68.6%) than *Apis* bees (S = 4, RA = 31.4%). As compared to field population of single widely managed bee, *A. mellifera* (RA = 16.7 %), the aggregate abundance of other eight wild species (RA = 83.3 %) was five times higher. Time-wise, the bee abundance was significantly highest at 11:00 hr (11.3±0.8 bees/30 net-sweeps) followed by 14:00 hr (8.3±0.8 bees/ 30 net-sweeps), 0800 hr (5.8±0.5 bees/ 30 net-sweeps) and 17:00 hr (5.8±0.7 bees/ 30 net-sweeps), with latter two statistically at par with each other.

The Sub-mountain Undulating zone revealed lower species richness (S= 8) than in Central Plain zone due to absence of *X. aestuans* (Table 1). *M. lanata* was most abundant (13.3±1.3/ 30 net sweeps, RA = 21.4%) followed by *M. bicolor* (11.5±1.0/ 30 net sweeps, RA = 18.5%), and both were statistically at par with each other. *A. cerana was* found minimum in numbers (0.8±0.3/ 30 net sweeps, RA = 1.3%). As compared to Central Plain zone, lower species richness and higher collective abundance (S = 6, RA = 60.1%) of Apidae members was recorded in this zone; however, the species richness of megachilids remained unchanged, but with lower collective abundance (S = 2, RA = 39.9%) than in central plain. Though the species richness of Non-*Apis* and *Apis* bees was at par (S = 4), but the former group of bees greatly outnumbered (RA = 62.4%) the latter one (RA = 37.6%). In comparison to *A. mellifera* (RA = 16.6 %), the aggregate abundance of seven other wild species (83.4 %) was 5.02 times higher. Time-wise, significantly highest and lowest pollinator activity was recorded at11:00 hr (10.8±0.8 bees/ 30 net-sweeps) and at 17:00 hr (4.6 0.5 bees/30 net-sweeps), respectively.

The species richness in Western zone (S = 8) was lower than in Central Plain (Table 1). *M. lanata* was the most abundant species (10.2±1.2/ 30 net sweeps, RA = 21.7%) followed by *M. bicolor* (9.6±1.1/ 30 net sweeps, RA = 20.3%) and *A. mellifera* (9.1±0.4/ 30 net sweeps, RA = 19.5%), with all three statistically at par among themselves. Field population of *A. florea* was significantly lowest (1.1±0.4/ 30 net sweeps, RA = 2.2%). As compared to Central Plain zone, lower species richness and higher collective abundance (S = 6, RA = 61.1%) of Apidae was recorded in this zone; however, the species richness of megachilids remained unchanged (S = 2), but with lower collective abundance (RA = 39.8%). The species richness of Non-*Apis* and *Apis* bees was at par (S = 4), but the former group greatly outnumbered (RA = 60.1%) the latter one (RA = 39.8%). Among the three zones surveyed, the managed bee pollinator, *A. mellifera,* was most abundant in Western zone (RA = 19.5 %), whereas in this zone the aggregate abundance of seven other species (RA = 80.5 %) was 4.13 times higher to that of *A. mellifera*. Time-wise, significantly highest and lowest bee activity was recorded at 11:00 hr (7.6±0.7 bees/ 30 net-sweeps) and 08:00 hr (2.7±0.4 bees/ 30 net-sweeps), respectively.

Overall, megachilids were the major pollen vectors in pigeon pea centred agro-ecosystem with mean relative abundance ranging from 39.9–43.6 % over these zones, which emphasized the significance of non-*Apis* species in providing crucial ecosystem service. However, the mean abundance of *A. mellifera* ranged between 16.6–19.5 % as compared to other wild bee species (RA = 80.5–83.4%) on pigeon pea. The highest and lowest proportion of non-*Apis* bees were recorded in central plain (RA = 68.6%) and Western zone (RA = 60.1%), respectively. However, the Sub-mountain undulating zone revealed comparatively balanced proportion of both *Apis* and non-*Apis* bees. Significant differences in temporal bee abundance were recorded with the highest bee activity at 11:00 hr (9.9±0.5/ 30 net sweeps) followed by 14:00 hr (7.1±0.4/ 30 net sweeps). However, differences in the bee abundance at 08:00 hr (4.5±0.3/ 30 net sweeps) and 17:00 hr (5.0±0.4/ 30 net sweeps) were non-significant. The differences in bee species richness, abundance and distribution in various zones were apparently influenced by local resource abundance, weather, ecological disturbances and surrounding floral matrix. The present findings are in line with the earlier reports of Kumar and Salunke (2009) who reported the *Megachile* sp. as the most abundant visitor (42.1 % relative abundance) on *C. cajan* blooms in Haryana, Gupta and Yadav (2001) documented *Megachile* species as the major apoidean pollinators of *C. cajan* in eastern Rajasthan and Punjab. Kumar and Salunke (2009) documented maximum diurnal abundance of *Megachile* sp. between 10:00 and 12:00 hr and lowest, either between 06:00 and 08:00 hr or 16:00 and 18:00 hr on *C. cajan*. Singh *et al*. (2003) reported highest number of *A. dorsata*, *A. florea*, *A. mellifera* and *A. cerana* between 11:00 to 14:00 hr on *C. cajan.*

Species diversity : Bee species diversity is a multi-dimensional property of any ecosystem, and diversity indices aim to describe general properties of communities allowing us to compare different regions, taxa, and trophic levels. Therefore, they are of fundamental importance for environmental monitoring and conservation. Using multiple indices can provide greater insight into the interactions in a system, and further as there is no consensus about which indices are more appropriate and informative (Chiarucci *et al*., 2011, Morris *et al*., 2014), in the present study twelve commonly used diversity indices (Chiarucci *et al*., 2011) were followed for comparing bee diversity in various zones and at different times of the day (Table 2). The Shannon-Wiener diversity index revealed superior diversity for Central Plain zone (H' = 2.009 at 11:00 hr) and Sub-mountain Undulating zone (H' = 1.967 at 11:00, H' = 1.849 at 08:00 and H' = 1.844 at 14:00 hr). The lowest value for the same was recorded in Western zone (H' = 1.351 at 0800 hr). The Brillouin index gave highest diversity value to bee community in Central Plain zone (HB = 1.972 at 1100 hr) followed by Sub-mountain undulating zone (HB = 1.929 at 1100 hr), while lowest for Western zone (HB = 1.290 at 0800 hr). The Inverse Simpson's Index also revealed highest diversity value for Central Plain zone [1/D(S) = 6.706 at 1100 hr] followed by Sub-mountain undulating zone [1/D(S) = 6.653 at 11:00 hr], while the lowest for Western zone [1/D(S) = 3.786 at 08:00 hr]. In general, of the twelve diversity indices calculated, eight gave highest diversity value to the Central Plain zone (at 11:00 hr or 08:00 hr), and the lowest diversity value to Western zone (at 08:00 hr or 17:00 hr). The Kendall coefficient of concordance (*W*), was significant (W=0.231, p=0.01, d.f.=11) which clearly indicated a

Table 1 : Diurnal relative abundance of bee pollinators visiting *Cajanus cajan* blooms in various agroclimatic zones of Punjab

Pollinator species	Mean number of pollinators per 30 net sweeps															
	Central Plain zone					Sub-mountain Undulating zone					Western zone					Overall mean
	08:00hr	11:00hr	14:00hr	17:00hr	Mean	08:00hr	11:00hr	14:00hr	17:00hr	Mean	08:00hr	11:00hr	14:00hr	17:00hr	Mean	
Apis mellifera	11.4±0.7 (3.5)	15.0±1.2 (4.0)	12.4±0.9 (3.6)	8.0±0.8 (3.0)	11.7±0.6 (3.5)	10.9±1.0 (3.4)	12.8±0.8 (3.7)	9.1±0.4 (3.2)	8.3±0.6 (3.0)	10.3±0.5 (3.3)	8.4±0.6 (3.1)	10.8±0.8 (3.4)	9.6±0.7 (3.2)	7.8±0.5 (2.9)	9.1±0.4 (3.2)	10.4±0.7 (3.3)
Apis cerana	2.1±0.8 (1.6)	1.4±0.6 (1.5)	0.0±0.0 (1.0)	0.0±0.0 (1.0)	0.9±0.3 (1.3)	0.0±0.0 (1.0)	3.1±1.1 (1.9)	0.0±0.0 (1.0)	0.0±0.0 (1.0)	0.8±0.3 (1.2)	0.0±0.0 (1.0)	4.4±1.1 (2.2)	3.5±0.7 (2.1)	0.0±0.0 (1.0)	2.0±0.5 (1.6)	1.2±0.4 (1.4)
Apis dorsata	5.8±0.6 (2.6)	11.3±1.0 (3.5)	8.0±0.7 (3.0)	5.0±0.6 (2.4)	7.5±0.6 (2.9)	6.8±0.5 (2.8)	13.4±0.6 (3.8)	10.8±0.6 (3.4)	5.8±0.3 (2.6)	9.2±0.6 (3.1)	4.0±1.0 (2.1)	9.6±0.7 (3.2)	7.1±0.6 (2.8)	5.3±0.6 (2.5)	6.5±0.5 (2.7)	7.7±0.8 (2.9)
Apis florea	2.4±0.6 (1.8)	5.3±0.7 (2.5)	0.0±0.0 (1.0)	0.0±0.0 (1.0)	1.9±0.4 (1.6)	2.3±0.6 (1.7)	6.5±1.1 (2.7)	3.5±0.9 (2.0)	0.0±0.0 (1.0)	3.1±0.6 (1.9)	0.0±0.0 (1.0)	4.3±0.9 (2.2)	0.0±0.0 (1.0)	0.0±0.0 (1.0)	1.1±0.4 (1.3)	2.0±0.6 (1.6)
Ceratina binghami	8.6±0.6 (3.1)	12.0±0.7 (3.6)	9.4±0.8 (3.2)	5.4±0.9 (2.4)	8.8±0.6 (3.1)	8.0±0.7 (3.0)	11.0±0.7 (3.5)	5.6±0.4 (2.6)	2.5±0.6 (1.8)	6.8±0.6 (2.7)	6.3±1.3 (2.6)	8.4±0.8 (3.0)	8.0±0.6 (3.0)	6.0±1.0 (2.6)	7.2±0.5 (2.8)	7.6±0.6 (2.9)
Xylocopa aestuans	0.0±0.0 (1.0)	6.5±0.8 (2.7)	3.9±0.6 (2.2)	0.0±0.0 (1.0)	2.6±0.6 (1.7)	0.0±0.0 (1.0)	0.0±0.0 (1.0)	0.0±0.0 (1.0)	0.0±0.0 (1.0)	0.0±0.0 (1.0)	0.0±0.0 (1.0)	0.0±0.0 (1.0)	0.0±0.0 (1.0)	0.0±0.0 (1.0)	0.0±0.0 (1.0)	0.9±0.9 (1.2)
Xylocopa fenestrata	9.4±0.7 (3.2)	7.3±0.7 (2.9)	4.5±0.9 (2.3)	3.4±0.9 (2.0)	6.1±0.6 (2.6)	9.1±0.7 (3.2)	10.8±1.0 (3.4)	6.0±0.7 (2.6)	3.0±0.9 (1.9)	7.2±0.7 (2.8)	5.3±0.9 (2.4)	0.0±0.0 (1.0)	0.0±0.0 (1.0)	0.0±0.0 (1.0)	1.3±0.5 (1.4)	4.9±1.8 (2.2)
Megachile bicolour	4.9±1.3 (2.3)	20.0±1.1 (4.6)	17.1±1.1 (4.2)	16.3±1.2 (4.1)	14.6±1.2 (3.8)	4.0±1.2 (2.1)	17.3±0.8 (4.3)	13.1±0.9 (3.7)	11.6±0.7 (3.5)	11.5±1.0 (3.4)	0.0±0.0 (1.0)	14.8±1.3 (3.9)	12.3±1.1 (3.6)	11.3±1.2 (3.5)	9.6±1.1 (3.0)	11.9±1.5 (3.4)
Megachile lanata	7.5±0.7 (2.9)	23.1±0.6 (4.9)	19.3±1.2 (4.5)	14.0±0.6 (3.9)	16.0±1.1 (4.0)	5.0±1.2 (2.3)	22.4±1.3 (4.8)	15.6±0.9 (4.1)	10.3±0.7 (3.3)	13.3±1.3 (3.6)	0.0±0.0 (1.0)	16.4±1.1 (4.1)	14.0±1.2 (3.9)	10.4±1.0 (3.3)	10.2±1.2 (3.1)	13.2±1.7 (3.6)
Mean	5.8±0.5 (2.4)	11.3±0.8 (3.3)	8.3±0.8 (2.8)	5.8±0.7 (2.3)	7.8±0.4 (2.7)	5.1±0.5 (2.3)	10.8±0.8 (3.2)	7.1±0.6 (2.6)	4.6±0.5 (2.1)	6.9±0.4 (2.6)	2.7±0.4 (1.7)	7.6±0.7 (2.7)	6.1±0.6 (2.4)	4.5±0.6 (2.1)	5.2±0.4 (2.2)	-
Overall Mean	4.5±0.3 (2.1)	9.9±0.5 (3.1)	7.1±0.4 (2.6)	5.0±0.4 (2.2)												

Data values represent pooled mean for 2012 and 2013 ± S.E.$_m$; Figures in parentheses are the means of $\sqrt{n+1}$ transformation; LSD (p=0.05) Pooled; Location (0.1); Time (0.1); Location x Time (0.1); Pollinator (0.1); Location x Pollinator (0.2); Time x Pollinator (0.2); Location x Time x Pollinators (0.4)

Table 2 : Mean diurnal diversity indices of bee pollinators visiting *Cajanus cajan* blooms in various agroclimatic zones of Punjab

Zone	Time	S	N	D(Mg)	D(Mn)	HB	H'	1/D(S)	U (McIn)	1/d	DC	E of H'	E (McIn)	D	D (McIn)
Central	0800h	8	416	1.161	0.392	1.888	1.825	6.605	163.0	4.571	9.06	0.878	0.941	0.122	0.640
Plain	1100h	9	814	1.194	0.315	1.972	2.009	6.706	315.4	4.400	10.0	0.914	0.919	0.086	0.635
	1400h	7	596	0.939	0.287	1.769	1.810	5.570	253.5	3.870	8.14	0.930	0.924	0.070	0.599
	1700h	6	416	0.829	0.294	1.594	1.642	4.628	194.2	3.200	7.25	0.916	0.901	0.084	0.561
Sub-	0800h	7	368	1.010	0.359	1.790	1.849	6.005	151.2	4.230	8.16	0.950	0.876	0.050	0.574
mountain	1100h	8	777	1.052	0.287	1.929	1.967	6.653	302.3	4.341	9.06	0.946	0.945	0.054	0.634
Undulating	1400h	7	510	0.962	0.310	1.792	1.844	5.903	210.9	4.080	8.14	0.948	0.943	0.052	0.614
	1700h	6	331	0.862	0.330	1.600	1.658	4.857	151.1	3.559	7.25	0.925	0.919	0.075	0.575
Western	0800h	4	191	0.571	0.289	1.290	1.351	3.786	98.9	2.851	5.63	0.974	0.965	0.026	0.520
	1100h	7	548	0.951	0.299	1.798	1.669	5.909	226.5	4.183	8.14	0.857	0.943	0.143	0.613
	1400h	6	436	0.823	0.287	1.669	1.715	5.302	190.3	3.893	7.25	0.957	0.952	0.043	0.592
	1700h	5	325	0.692	0.277	1.509	1.251	4.664	151.3	3.611	6.40	0.777	0.967	0.223	0.566

Kendall coefficient of concordance, W = 0.231; *Data values represent mean for the years 2012 and 2013; **Definitions of symbols: S, Species richness; N, Total number of bee individuals; D(Mg), Margalef's index; D(Mn), Menhinick's index; HB, Brillouin's index; H', Shannon-Weiner index; 1/D(S), Inverse Simpson's index; U(McIn), McIntosh's U index; 1/d, Inverse Berger-Parker's index; DC, Cuba's index; E of H', Shannon evenness; E(McIn), McIntosh evenness; D, Southwood dominance index; D(McIn) McIntosh dominance index

reasonably high consensus among the indices. Averaging the ranks of the zones (using 12 indices at 4 times of the day, the total number of bee individuals and species richness in each zone), the most diverse zone was Sub-mountain undulating zone, while least diverse was Western zone. Timewise, the most diverse bee communities were recorded at 1100 hr, followed by 14:00 and 08:00 hr, with lowest community diversity at 1700 hr.

The highest bee diversity in Sub-mountain undulating zone was attributed to the occurrence of multiple ideal microhabitats and availability of rich natural vegetation for bee species throughout the year. Further, the presence of undisturbed forest land and orchards in Sub-mountain undulating zone provided ideal nesting zones for survival and multiplication of solitary bees and feral colonies of bees. The lowest bee community diversity in Western zone was attributed to inundation of agricultural and surrounding land as a result of rising groundwater table, mono-cropping (Cotton or Paddy) and higher anthropogenic pressure leading to destruction of nesting zones of solitary bees. Further, the pesticide consumption in Punjab increased from 3200 metric tonnes in 1980-81 to 5970 metric tonnes (technical grade) in 2005-06 and the cotton belt in Punjab (Western zone), alone accounts for nearly 75 % of this total consumption (Tiwana *et al*., 2007). This indiscriminate use of pesticides on cotton and paddy in Western zone might be a potential cause of reduced bee abundance and overall diversity in this zone.

The results also offer a platform for the application of the aspect of risk assessment that has not been applied earlier to pollination for better incorporation of pollinators' services. These observations serve as potential basis for initiating habitat management for conservation and augmentation of the native bee species struggling for their survival.

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