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Influence of Annual Ornamental Flowers (*Asteraceae*) on the Relative Abundance of Honey Bee Species in the Hot Semi-arid Environment

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Abstract: Honey bees are an important component of the agricultural ecosystem and provide valuable pollination service. Cultivation of annual ornamental flowering plants is being recognized as a suitable options to compensate for the loss of natural floral resources for conserving the declining population of the honey bee. The capitulum/ inflorescence of commonly grown annual ornamental flowers of the Asteraceae family differs in morphology. The morphology of flowers affects the abundance and diversity of bee species. Therefore, the present study was conducted with an aim to study the influence of commercially cultivated flowering plants of India (*Calendula officinalis, Chrysanthemum indicum, Glebionis segetum, Tagetes patula* and *Tagetes erecta*) on honey bee diversity and abundance in the hot semi-arid environment. The capitulum of these plant species differed significantly in length and diameter of the corolla tube. It was observed that dwarf (*Apis florea*) and giant (*Apis dorsata*) honey bee were the most common visitors, however, the abundance of both bee species was significantly different ($P<0.01$) on different plant species. Plants with relatively longer (15.25-18.9 mm) and wider corolla tube *(Tagetes erecta* and *Tagetes patula)* were visited by both the bee species. However, plants having short (5.0-6.5 mm) and narrow (1.0-1.33 mm) corolla tubes *(Calendula officinalis, Chrysanthemum indicum, Glebionis segetum)* were dominantly visited by only *Apis florea*. Therefore, the cultivation of *Tagetes patula* and *Tagetes erecta* may enhance the population and conservation of both *Apis florea* and *Apis dorsata*, while *Calendula officinalis, Chrysanthemum indicum,* and *Glebionis segetum* may only enhance the population of *Apis florea* in the arid and semi-arid zone of India.

Key words: Flower size, *Apis dorsata, Apis florea*, arid region, foraging behavior.

Honey bees are a key biotic component of the agro-ecosystem. They provide valuable ecosystem service in the form of pollination to the agricultural and horticultural crops for seed and fruit set (Chakrabarty and Shama, 2007; Das *et al.,* 2011). They also provide additional income to the farmers in the form of honey and wax. The annual economic value of pollination services has been estimated at over US$ 200 billion around the world (Gallai *et al.,* 2009). About two-third of the leading global crop plants get benefited from animal-mediated pollination and among these animals, bees are primary pollinators (Klein *et al.,* 2007). However, there is clear evidence of the recent decline in bees colonies, and parallel decline in the population of plant species that are dependent on bees for outcrossing (Biesmeijer *et al.,* 2006). The decline in honey bee colonies has been reported in the different parts of the world (Potts *et al.,* 2010) and also in India (Sihag 2014). If the declining trend continues, it may reduce seed and fruit production from many outcrossing crops.

One of the major causes which have often been linked with pollinators decline is degradation and loss of natural habitats and floral resources due to developmental activities, e.g., intensification of agriculture and urbanization (Potts *et al.,* 2010; Hülsmann *et al.,* 2015). Therefore, enhancing the availability and diversity of floral resources is one of the major strategies for conserving honey bee population and diversity. Garden flowering plants are being frequently recognized as alternative options for substituting dwindling diversity of natural forage sources for bees and other flower-visiting insects (Garbuzov *et al.,* 2013; Hülsmann *et al.,* 2015). Bringing some part of agricultural land under floriculture may be a good option to enhance population and diversity of pollinators especially honey bee.

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In India, floriculture is practiced on about 70581 ha agricultural land (HSG, 2017). Steady demand for ornamental flowers in the global and local market, provide an opportunity to further increase the area under ornamental flower production. Increasing trends in area and production of flowers have been observed since 2003-04 onwards in the country. The total area and production of flowers and aromatic have increased from 0.1 million hectares in 2001-02 to 0.94 million hectares in 2016-17 (HSG 2017). However, the arid and semi-arid regions of India share very less area (<1%) under floriculture. Due to high income, increasing demand for flower and sufficient availability of cultivated land, floriculture in this region has a scope for growth in the future. However, variation has been reported among garden flowering plants in terms of attractiveness to bees and other flower-visiting insects (Garbuzov *et al.,* 2013). This is mainly due to morphological differences in the size of flower and foragers (Suzuki, 1994; Sihag and Rathi, 1992; Stout, 2000; Garbuzov *et al.,* 2013; Marzinzig *et al.,* 2018). Therefore, the introduction of ornamental flowering plants and extending areas under floriculture may enhance the abundance of pollinators but may also change the diversity of pollinators.

Annual ornamental flowering plant species of the Asteraceae family i.e. marigold (*Tagetes patula* and *Tagetes erecta*), chrysanthemum (*Chrysanthemum indicum* and *Glebionis segetum*), gaillardia (*Gaillardia* sps.) and pot marigold (*Calendula officinalis)* are major commercially cultivated species in India. Out of the total area under floriculture in India, marigold occupies the largest area (24.5%) followed by jasmine (5%), chrysanthemum (1.7%), orchid (0.3%) and gaillardia (0.02%) (HSG 2017). However, the capitulum of these plant species differs in the size of disc florets, therefore, it might also differ in attracting different pollinators. Limited work has been reported on the preferences of different pollinators on these plant species especially in the hot semi-arid environment of India. Differences in the size of disc florets in these plants provide an opportunity to observe the impact of flower size on abundance and diversity of bee species. With this background, the present study was undertaken to observe differences in behavior and preferences of pollinators on the most commonly cultivated plant species i.e. *Tagetes patula, Tagetes erecta, Calendula officinalis, Chrysanthemum indicum* and *Glebionis segetum*. All these plants belong to the Asteraceae family and differ in the size of disc florets. The objective of the study was to quantify diversity, abundance and preferences of pollinators and their diurnal variation, on the morphologically different type of inflorescences in a traditionally uncultivated area to enhance the population of bees and adoption of floriculture in this area. We hypothesized that (i) these plant species will attract honey bee species and (ii) interaction between flower and pollinators depends on the size of disc florets.

Materials and Methods

Study site and experimental setup

The study was undertaken during the winter season in 2015-16 and 2016-17 at the research farm of ICAR-Central Arid Zone Research Institute, Regional Research Station, Pali. The research station is spread over 450 ha land (25°47′- 25°49′ E and 73°17′-73°18′ N) at 217-220 m above mean sea level. The region receives about 420 mm average annual rainfall with 42°C and 7°C annual maximum and minimum mean temperature, respectively. Foraging behavior of honey bee species was observed on five garden flower species of the Asteraceae family which were first time cultivated in the study area. The selected plant species were *Calendula officinalis*, *Chrysanthemum indicum*, *Glebionis segetum, Tagetes patula* and *Tagetes erecta.* Each species were planted on a separate bed of size 3*6 m with 0.45*0.45 m plant spacing in November 2015 and 2016. Beds were separated by one meter from each other. A circular area of 0.1 m^2 was randomly demarcated at three places in each bed by a ring of the paper sheet for taking observations on the foraging behavior of insect foragers.

Observation and statistical analysis

The observation was started when plants were in full bloom during January to February 2016 and 2017. The observation was taken at 7-10 days interval. During each day of observation, data was recorded in four shifts of (8:30-9:30; 11:30-12:30; 13:30-14:30 and 15:30-16:30 h). In each shift, type of foragers that visited on capitula and their number was recorded visually for 10 minutes within each demarcated area on each plant species. Thus, a total of 120 minutes of visual observation were

taken on each day of observation on each plant species. The bees were visually identified by morphological characters. The number of days visited by honey bees on single capitulum after its anthesis was also recorded on 10 tagged capitula of each species. Length and diameter of corolla tube of ray and disc florets were recorded with scale and digital caliper respectively in 20 randomly plucked full-bloomed capitula of each plant species. The relative abundance RA of species was calculated by following formula RA(%)=(n*100)/(N) where n is the number of individuals of *A. florea* or *A. dorsata* and N is total number of *A. florea* and *A. dorsata* visiting on the given flower during observation. Duncan Multiple Range Test (DMRT) was carried out to compare the means of the observation at a 95% confidence level.

Results and Discussion

Diversity and foraging behavior

Two species of honey bee *Apis dorsata* and *Apis florea* were the most dominant foragers on the capitulum during the entire period of the study (Fig. 1). Other insect's visitors were syrphid fly and butterfly but were less frequent, therefore, numbers of these insects were not used in the calculation and comparison was made only between *Apis dorsata* and *Apis florea.* The capitulum of all the selected plant species began anthesis with the opening of the outer single whorl of ray florets followed by multiple whorls of disc florets. Foragers of *Apis dorsata* and *Apis florea* started foraging after anthesis of disc flower sequentially from outer whorl to inner whorl on the capitulum of all the plant species. Bee visited flowers to get pollen and nectar (sources of carbohydrates, proteins, lipids, and micronutrients) for survival, reproduction, and resilience to stress (Vaudo *et al.,* 2015). In the Asteraceae family, nectar is secreted from floral nectaries (annular, multicellular outgrowths) which are formed on the top of the inferior ovary and surround the style base (Mani and Saravanan, 1999; Wist and Davis, 2006). However, all florets do not produce nectar. Disc florets are the main source of pollen and nectar to the honey bee, while ray florets are mostly sterile and do not produce pollen grain and nectar (Mani and Saravanan, 1999; Wist and Davis, 2006). Furthermore, generally, florets in the capitulum start anthesis from outer whorl to inner whorl (Wist and Davis, 2006). Thus in this study, both the species of honey bee started foraging after anthesis of the main source of pollen and nectar i.e. disc florets and followed the pattern of foraging from outer whorl of disc floret to inner whorl.

Both honey bee species showed diurnal as well as temporal variation in the rate of visit during both the years of observation (Fig. 2). The lowest visitation rate was during the morning (9:30-10:30 h) and evening (15:30-

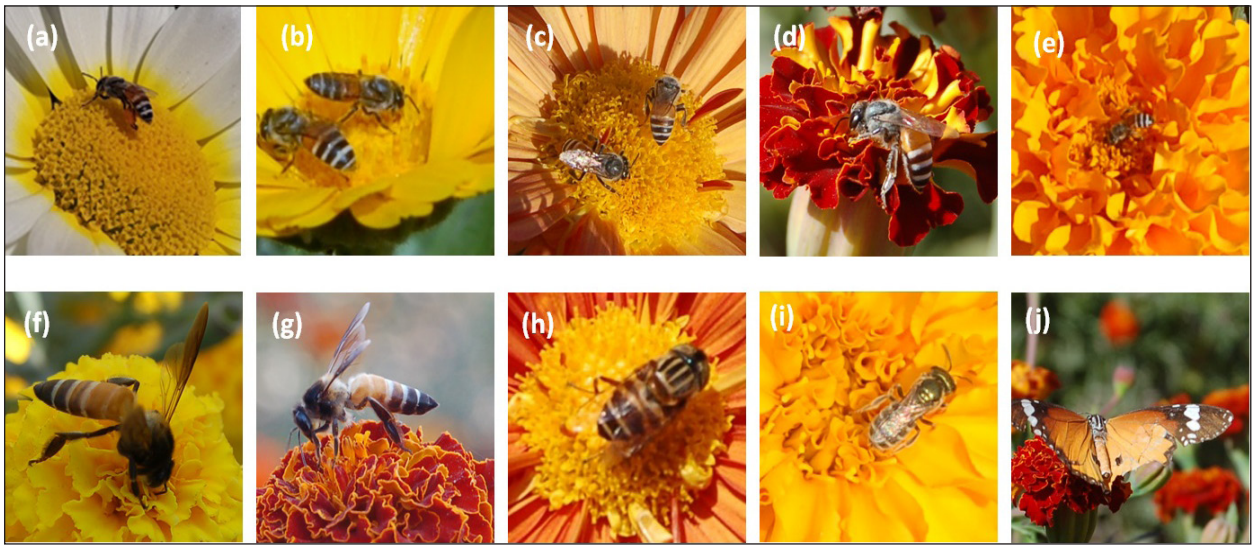

Fig. 1. Insect visitors on ornamental flowering plants: (a), (b), (c), (d), (e) and (f) Apis florea foraging on Glebionis segetum, Calendula officinalis, Chrysanthemum indicum, Tagetes patula and Tagetes erecta respectively; (g) and (h) Apis dorsata foraging on Tagetes patula and Tagetes erecta respectively; (i) syrphid fly foraging on Chrysanthemum indicum; (j) megachile bee foraging on Tagetes erecta and (k) butterfly foraging on Tagetes patula.

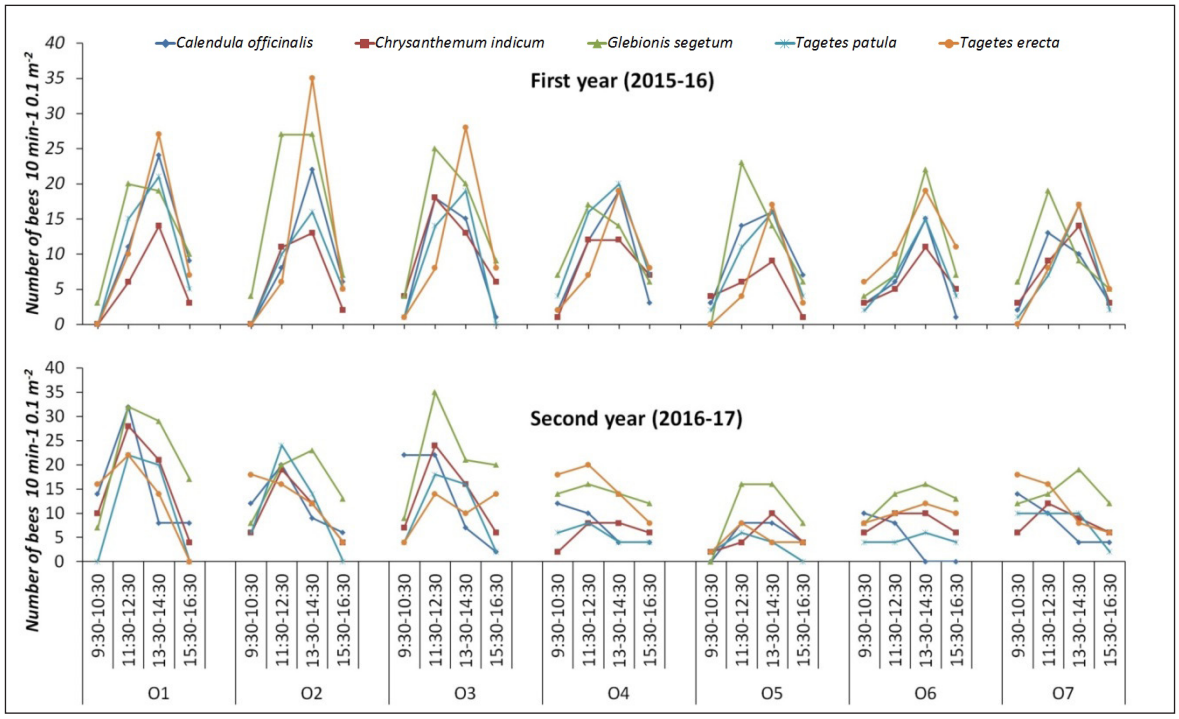

Fig. 2. Temporal variation in visitation rate of honey bee species (Apis florea +Apis dorsata) on the capitula of ornamental flowering plants. 01 to 07 are observations taken during experimental period.

16:30 h), while, highest was during midday (11:30-14:30 h) (Fig. 2). Days of foraging per capitulum after its anthesis varied significantly among the plant species. It ranged from 7.0 to 17.5 days and was highest on the capitulum of *Tagetes patula.* Days of foraging per capitulum among the plant species were in the order of *Chrysanthemum indicum* (7.0 days) ≤ *Calendula officinalis* (7.8 days) ≤ *Glebionis segetum* (9.0 days) < *Tagetes erecta* (14.7days) ≤ *Tagetes patula* (17.5 days) (Table 2). Diurnal and temporal variation in the visitation rate of bee has been well studied. This variation is due to variation in weather, rate of anthesis and volume of nectar in corolla tube (Wist and Davis, 2006). In the present study, all observations were recorded on a bright sunny day to minimize the impact of cloudy weather. Therefore, the diurnal variation in the visitation rate might be primarily due to diurnal differences in the quantity of nectar in the corolla tube of disc florets. It was reported that, regardless of the stage of floret development, daily, nectar volumes tend to be highest at midday and low in morning and evening (Wist and Davis, 2006).

Relative abundance and preferences

All the plant species in the study attracted honey bees, however, differed significantly in attracting *Apis dorsata*, *Apis florea* and the total number of honeybee (*Apis dorsata* + *Apis florea*) per unit area (Table 2). The total number of honeybee visited per unit area was highest on *Glebionis segetum* (377.2±6.3) followed *Tagetes erecta* (267.1±10) < *Chrysanthemum indicum* (252.7±13.3) ≤ *Calendula officinalis* (230.6±4.7) and *Tagetes patula* (219.2±8.0) (Table 2 and Fig. 3). Plant species were significantly different in corolla tube length and diameter (Table 1). Capitulum of *Tagetes patula* and *Tagetes erecta* had disc florets with long (15.25-18.9 mm) and wide (1.83-2.1 mm) corolla tube, while *Calendula officinalis*, *Chrysanthemum indicum*, *Glebionis segetum* had short (5.0-6.5 mm) and narrow (1.0-1.33 mm) corolla tube.

Irrespective of corolla tube length and diameter, *Apis florea* foraged on the capitulum of all the plant species and its relative abundance was 47.2% in *Tagetes erecta*, 53.6% in *Tagetes patula*, 97.3% in *Glebionis segetum*, 97.4% in *Chrysanthemum indicum* and 98.0% in *Calendula officinalis* (Table 2 and Fig 3). While, *Apis dorsata* foraged mainly on capitulum of plants with longer (15.25-18.9 mm) and wider corolla tube (1.83-2.10 mm) i.e. *Tagetes patula* (49.3%) and *Tagetes erecta* (56.5%) and was absent or rarely visited on plants with shorter (5.0-6.5 mm) and

Table 1. Morphological characteristics of inflorescence of ornamental flowering plants (Asteraceae)

Plants (Asteraceae)		Ray florets			Disc florets			
Botanical name	Common name	Color	Type of corolla	Legule length (mm)	Color	Type of corolla	Corolla tube length (mm)	Corolla tube diameter (mm)
Calendula officinalis	Pot marigold	Yellow and orange	Single ligule	22.2±0.4b	Yellow and orange	Corolla tube (five fused petals)	5.00±0.18a	1.05±0.13ab
Chrysanthemum indicum	Indian chrysanthemum	Red and yellow	Single ligule	23.4±1.5b	Yellow	Corolla tube (five fused petals)	6.25±0.3b	1.00±0.18a
Glebionis segetum	Corn marigold/ daisy	White and yellow	Single ligule	19.1±1.5a	Yellow	Corolla tube (five fused petals)	6.48±0.25b	1.33±0.22b
Tagetes patula	French marigold	Red and yellow	Single ligule	25.3±0.7c	Yellow	Corolla tube (five fused petals)	15.25±0.65c	1.83±0.17c
Tagetes erecta	African marigold	Yellow and orange	Single ligule	25.7±0.6c	Yellow and orange	Corolla tube (five fused petals)	18.90±0.89d	2.10±0.22c

Note: According to DMRT mean followed by different letter(s) in a column are significantly different (P=0.05).

narrower corolla tube (1.0-1.3 mm) i.e. *Calendula officinalis* (1.3%), *Chrysanthemum indicum* (2.8%) and *Glebionis segetum* (2.9%) (Table 1 and 2, Fig. 3).

Sajjad (2017) also reported that *Apis dorsata* do not visit the flowers which are most preferred by *A. florea*. Further, generally, *Apis dorsata* prefer flowers with high caloric rewards (Sihag and Rathi, 1992). The base of the corolla tube act as a reservoir of nectar and the amount of nectar production is positively correlated with flower size (Navarro and Medel, 2009; Tavares *et al.,* 2016). Length and diameter of the corolla tube is the most important factor affecting access to the nectar by a honey bee in the corolla tube. Bee with long proboscis length can easily access nectar from shorter as well as equivalent or longer length of corolla tube if corolla tube diameter is sufficiently wider (Suzuki 1994; Stout 2000; Courcelles *et al.,* 2013; Marzinzig *et al.,* 2018). Corolla

Table 2. Total number of bee and duration of visit on the capitula of ornamental flowering plants during the period of observations (2 years mean)

Plants (Asteraceae)	*Apis dorsata* (0.1 m^2)	*Apis florea* (0.1 m^2)	Total (0.1 m^2)	Duration of bee visit (day capitulum^{-1})	p value (*A. fl.* x *A. dor.*)
Calendula officinalis	4.6±0.1a (2.0%)*	226.1±4.7b (98.0%)	230.6±4.7a	7.8±1.0a	<0.001
Chrysanthemum indicum	6.6±0.5a (2.6%)	246.1±13.8c (97.4%)	252.7±13.3b	7.0±0.8a	<0.001
Glebionis segetum	10.0±2.6a (2.7%)	367.2±8.9d (97.3%)	377.2±6.3c	9.0±1.8a	<0.001
Tagetes patula	101.8±7.6b (46.4%)	117.4±2.5a (53.6%)	219.2±8.0a	17.5±2.1c	0.023
Tagetes erecta	140.9±12.8c (52.8%)	126.2±3.2a (47.2%)	267.1±10b	14.7±2.2b	0.124

*Relative abundance within parenthesis.
Note: According to DMRT mean followed by different letter(s) in a column are significantly different (P=0.05).

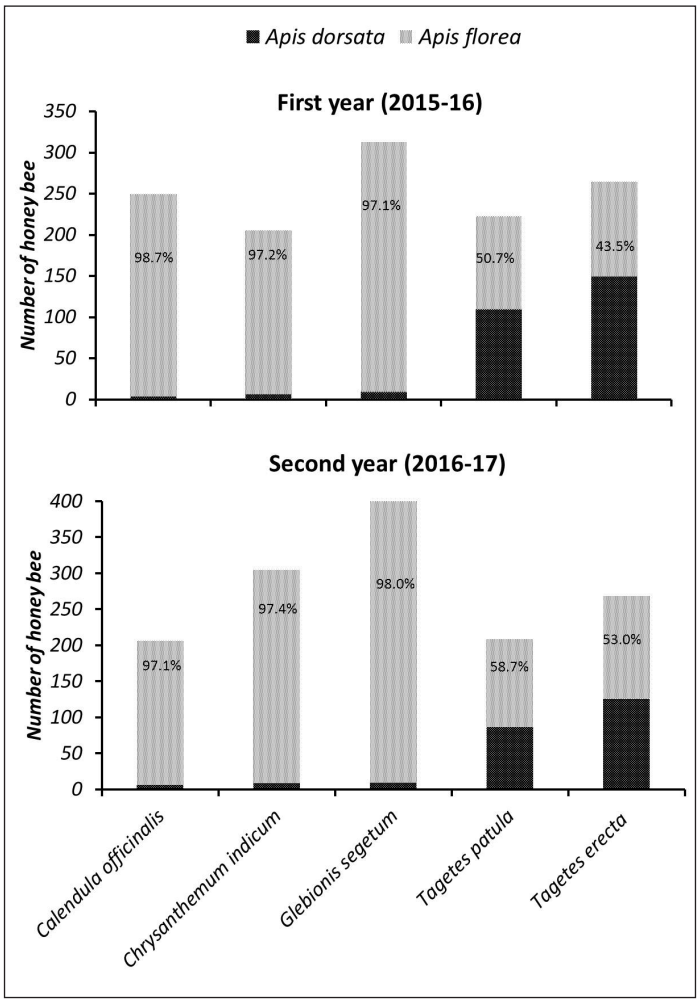

Fig. 3. Relative abundance of Apis dorsata and Apis florea on the capitula of ornamental flowering plants during entire observation.

diameter was found as a main morphological determinant of visit rates of honey bees, which is significantly higher on the wider flowers (Suzuki, 1994; Courcelles *et al.,* 2013). Probosic length and prementum width of *Apis dorsata* varied from 6.54-6.56 mm and 0.83-0.91 mm respectively while in *Apis florea* it varied from 3.45-3.57 mm and 0.56 mm (Niem and Trung, 1999). The proboscis size of *Apis dorsata* is larger and thicker than *Apis florea*. Therefore, *Apis florea* may have easy access to nectar in the narrow corolla tube of *Glebionis segetum, Chrysanthemum indicum* and *Calendula officinalis* as compared to *Apis dorsata*.

The annual ornamental flowers *Calendula officinalis, Chrysanthemum indicum, Glebionis segetum, Tagetes patula* and *Tagetes erecta* were the good attractor of *Apis florea* and *Apis dorsata*. These flowers may be helpful in enhancing the population and colonies of these honeybee species. The study found a significant effect of the size of the corolla tube on attracting large and small size honeybee species. As a result, *Apis florea* preferred to forage on capitula of all the plants in the study, while *Apis dorsata* preferred to visit on capitula with a relatively wider and longer corolla tube. Thus, *Tagetes patula* and *Tagetes erecta* may help enhance the population and conservation of both *Apis florea* and *Apis dorsata*, while *Calendula officinalis, Chrysanthemum indicum* and *Glebionis segetum* may only enhance the population of *Apis florea*.

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