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## Influence of floral nectar quantity and quality on honey bee foraging activity in onion

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### Abstract

The experiment was conducted to study the relationship between the onion floral nectar characteristics and honey bee foraging activity. The role of nectar characteristics in influencing their attractiveness to honey bees was evaluated in three cultivars of onion. Onion varieties with more nectar, TSS and caloric rewards attracted large number of honey bees (*Apis cerana*, *Apis florea*, *Apis dorsata* and *Tetragonula iridipennis*). Arka Pragathi variety was found to produce significantly superior quantity and quality of nectar, that attracted a large number of honey bee foragers. All the onion varieties studied produced the floral nectar which is hexose dominant, rich in fructose and glucose sugars. The honey bee foraging populations showed a significant positive correlation with average nectar volume and TSS (except *Apis dorsata* for nectar volume), thus honey bee foragers were found to be guided to flowers mainly because of their nectar quantity and quality.

**Keywords:** *Allium cepa*, onion, nectar sugars, honey bee, pollination

### 1. Introduction

Plant pollinator interaction is a highly evolved mutualism where floral characters play a significant role in attracting suitable foragers. Plants attract their pollinators in many ways, e.g. by offering nectar, pollen, tissues, oil, heat, perfumes, copulation sites and nesting materials [26]. The most important floral rewards are nectar and pollen, which energize flight, acts as energy and protein source, brood food for foragers and contain a variety of compounds. Besides morphological parameters like flower colour, shape and type of inflorescence, physical and biochemical characteristics of nectar influence to a greater extent to their attractiveness to insects like bees [9]. It is well established that honey bees visit flowers to gather pollen and nectar.

Florets of onion inflorescence are not self-fertile [8]. Cross pollination is essential because of its protandrous nature, where pollen is shed two to three days before the stigma is receptive [15]. The pollen grains of onion are sticky and heavy in nature and hence not amenable for wind pollination, so insect pollinators play a huge role in pollination [10, 5]. Flowers signal their presence at both long and short distances, and to guide the movements of visitors, using a variety of floral attractants. Nectar not only acts as the potential energy reward provided by the flowers but also fulfils the water demands of its consumers. Concentration and composition of the floral nectar may give idea on taxonomic group and physiological needs of its floral visitors. The volume and total soluble solids of the nectar are the most important factors, influencing the attractiveness of the pollinators [9]. Nectar sugar concentrations below 20% (weight/weight) are typical for bat-pollinated plants due to their high water demands, medium concentrations in humming bird pollinated plants, whereas bee-pollinated plants generally have nectar sugar concentrations above 30% [17]. Floral nectar composition has been studied as the potential cause of bee preferences for certain onion flower visitors [24, 11]. Various chemical constituents present in the nectar are sugars, amino acids, lipids, phenols and antioxidants [4]. Onion nectar is reported to be very rich source of sugars [11] and pollinators like honey bees prefer specific sugar composition (sucrose, glucose and fructose) in the nectar [25]. Thus, the quality and quantity of the nectar provided by the flowers act as a significant parameter that affects the behaviour of pollinators to their energy needs [1].

Therefore, with the above background information the experiment was carried out to establish the relationship between the nectar quality, quantity and the honey bee visitation to flowers an extremely entomophilic vegetable crop onion, and study the pollinator preference among different onion cultivars, probable reasons for their preference.

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## 2. Materials and Methods

The study was conducted at the ICAR– Indian Institute of Horticultural Research, (IIHR), Bengaluru (12°58' N; 77°35' E), India.

### 2.1 Plant Material

Three popular onion cultivars *viz.*, 'Arka Pragathi', 'Arka Kalyan' and 'Arka Nikethan' were used in the study. The bulbs were planted on bed of size 3 x 3 m at a spacing of 30 cm during (January) 2016, cultivated in a randomized complete block design with three replicates for each cultivar. Standard package of practices was followed for crop husbandry. Pesticides were not applied during the flowering stage to encourage the Pollinators activity.

### 2.2 Foraging activity of honey bees

Foraging behaviour of honey bees was studied by visual observation. The number of honey bees visiting ten randomly selected umbels, each umbel in five minutes duration in each of the onion variety was recorded.

### 2.3 Nectar collection

To minimize nectar evaporation and prevent bee foraging, umbels were covered with paper bags. Nectar was collected 24 hours after flowers were fully bloomed. Nectar was collected from 20 florets in each umbel using a 2 ml micro pipette and subsequently stored at -20 °C in 1ml centrifuge tubes sealed with parafilm. Samples were frozen (- 20 °C) until they were analysed for sugar composition.

A second sample was analysed at the time of collection in the field for nectar volume and sugar concentration or total soluble solids (TSS). Nectar volume was determined with constant-bore micropipettes by measuring the length of the nectar column from 20 florets. Sugar concentration was measured by placing a drop of nectar on a Bellingham and Stanley 40-85% sugar hand refractometer and recording percentage of total dissolved solids.

Caloric reward/flower/day was calculated by assuming that 1 mg of sugar (irrespective of the type) yields 4 calorie or 16.74 joules of energy (Henrich 1975) by the formula:

$$\frac{\text{Nectar volume } (\mu\text{l}) \times \text{Concentration of nectar } (\%) \times 16.74}{100}$$

### 2.4 Extraction of sugar

Sugars were extracted by a slight modification of method described by Steppuhn and Wäckers (2004) [22]. Four microliter ( $\mu\text{l}$ ) of onion flower nectar extracted with warm 80% ethanol and made up the volume to 5 ml. Then the 5 ml extractant was evaporated to dryness by keeping it on a water bath at 80 °C and the residue was dissolved in 2 ml of 0.01% formic acid. The mixture was sonicated for 10 minutes followed by extraction with ethyl acetate for 2-3 times. Ethyl acetate was removed completely by keeping it on water bath at 60-70 °C for 30 minutes, then the volume was made up using mobile phase containing solvent A and solvent B in 1:1 ratio. Then it was filtered and injected to LC-MS/MS (Waters UPLC H class system fitted with TQD MS/MS system) for analysis.

### 2.5 LC and MS-MS conditions

The mobile phase consisted of solvent A: 80:20-acetonitrile: water and solvent B: 30:70-acetonitrile: water + 0.1% ammonium hydroxide. The initial concentration

composed of 100% solvent A, held for 1 minute. At 8 minutes, the gradient changed to 88% of solvent A and 12% of solvent B, held for 1 min and a linear gradient follow by 98% of solvent A and 2% of solvent B at 15 minutes, held for 0.5 minute. The system was then returned to the initial condition at 19 minutes and equilibrates for 1 minute before the next injection. The flow rate was 0.1 mL/minute and the analytical column is 2.1 X 100 mm UPLC BEH-Amide column (Waters, USA) with 1.7 $\mu\text{m}$  particles, protected by a vanguard BEH-Amide with 1.7 $\mu\text{m}$ . Guard column (Waters, USA) was used with column temperature at 25°C. The elution was monitored using a PDA detector and the UPLC column effluent pumped directly without any split into the TQD-MS/MS (Waters, USA) system, optimized for the analysis of sugars.

Sucrose-hexose index was worked out to characterise the relative proportions of sugars by weight within the nectar samples which was established by Baker and Baker (1983) [4], which is expressed as follows:

$$\frac{S}{G + F}$$

(Concentrations [weight/volume] of S = sucrose, G = glucose, F = fructose)

These authors define ratios < 0.1 as "hexose dominated", between 0.1 and 0.499 as "hexose rich", between 0.5 and 0.999 as "sucrose rich" and > 0.999 as "sucrose dominated" [4].

### 2.6 Data analysis

The data were analysed for statistical significance by analysis of variance (ANOVA), to determine whether differences between cultivars with respect to nectar production and foragers. The means were compared through the Fisher least significant difference test by using SPSS software. The results were significant at  $P < 0.05$  unless specified otherwise. Coefficient of correlation was worked out to study the relationship between the foraging population, nectar volume and TSS.

## 3. Results

### 3.1 Pollinator diversity in relation to varietal difference

Flowers of all studied the varieties of onion were found to be attractive to different kinds of floral visitors. *viz.*, syrphids, bumble bees, butterflies, moths, honey bees, halictid bees, Megachile species and wasps. Among them honey bees *viz.*, *pis cerana*, *Apis florea*, *Apis dorsata* and *Tetragonula iridipennis* were found to be major foragers. Among these four species, *A. florea* was the dominant pollinator followed by *A. cerana*, *T. iridipennis* and *A. dorsata*. A difference in attractiveness of honey bees to different onion varieties was also recorded. The density and abundance of pollinators were significantly different among varieties.

Arka Pragathi variety on an average attracted 4.14 of *A. florea*, 2.28 of *A. cerana*, 1.73 numbers of *T. iridipennis* and 0.11 of *A. dorsata* in five minutes (Table 1). Level of attractiveness of Arka Pragathi variety was found to be superior for *A. florea*. Preference of *A. florea* (3.08 and 3.12) and *A. cerana* (1.81 and 1.58) to Arka Kalyan and Arka Nikethan respectively, was found on par with each other. *A. dorsata* treated all the three varieties in the same manner.

**Table 1:** Mean number of honey bee foragers on different onion varieties

Varieties	No. honey bees/umbel/5min			
	<i>Apis cerana</i>	<i>Apis florea</i>	<i>Apis dorsata</i>	<i>Tetragonula iridipennis</i>
Arka Pragathi	2.28 <sup>a</sup>	4.14 <sup>a</sup>	0.11 <sup>a</sup>	1.73 <sup>a</sup>
Arka Kalyan	1.81 <sup>b</sup>	3.08 <sup>b</sup>	0.14 <sup>a</sup>	1.62 <sup>a</sup>
Arka Nikethan	1.58 <sup>b</sup>	3.12 <sup>b</sup>	0.11 <sup>a</sup>	0.12 <sup>b</sup>

Values followed by a similar lower-case letter within treatments are not significantly different according to Fischer LSD test at  $P < 0.05$ .

Arka Pragathi and Arka Nikethan were significantly different for number of *T. iridipennis* (1.73) and (0.12) respectively. Arka Kalyan (1.62) was found on par with Arka Pragathi (1.73) for *T. iridipennis*. The preference of Arka Nikethan variety by *A. cerana* (1.58), *A. florea* (3.12) was found similar to Arka Kalyan. Number of honey bees foraging on Arka Nikethan was significantly less than those on Arka Pragathi (Table 1).

### 3.2 Nectar Parameters

Onion varieties differed in the amount of nectar produced and total soluble solids in the nectar (Table 2). The volume of nectar ranged from 1.37 to 1.81  $\mu\text{l}$ / flower and TSS ranged from 53.60 to 63.80. Energy produced per flower/day ranged between 12.29 to 19.33 joules (Table 2).

Variety Arka Pragathi produced maximum amount of nectar per floret (1.81  $\mu\text{l}$ / floret) and was rich in sugar concentration (TSS-63.80) and thus, provides maximum energy rewards per floret (19.33 joules), followed by Arka Kalyan and Arka Nikethan. Arka Kalyan and Arka Nikethan were found to be on par with each other with respect to amount of nectar (1.44 and 1.37  $\mu\text{l}$ / floret) and energy (14.89 and 12.29 joules) produced (Table 2).

**Table 2:** Nectar volume, TSS and Energy rewards in three different onion varieties

Varieties	Nectar volume ( $\mu\text{l}$ ) per floret	TSS	Energy (joules)
Arka Pragathi	1.81 <sup>a</sup>	63.80 <sup>a</sup>	19.33 <sup>a</sup>
Arka Kalyan	1.44 <sup>b</sup>	61.80 <sup>a</sup>	14.89 <sup>b</sup>
Arka Nikethan	1.37 <sup>b</sup>	53.60 <sup>b</sup>	12.29 <sup>b</sup>

Values followed by a similar lower-case letter within treatments are not significantly different according to Fischer LSD test at  $P < 0.05$ .

### 3.3 Nectar Composition

The amount of sugar available in nectar of each flower is dependent on nectar volume and total soluble solids. The

**Table 3:** Nectar composition on different onion varieties

Varieties	Sugars- mg/g of nectar												
	Ribose	Arabinose	Xylose	Fucose	Glucose	Fructose	Galactose	Mannose	Inositol	Sorbitol	Sucrose	Maltose	S/(G+F)
Arka Pragathi	1.870 <sup>a</sup>	0.103 <sup>a</sup>	0.008 <sup>b</sup>	0.001 <sup>b</sup>	10.999 <sup>a</sup>	20.339 <sup>a</sup>	0.026 <sup>a</sup>	1.269 <sup>a</sup>	0.080 <sup>a</sup>	0.333 <sup>a</sup>	0.151 <sup>a</sup>	0.014 <sup>a</sup>	0.0048 <sup>a</sup>
Arka Kalyan	1.618 <sup>b</sup>	0.056 <sup>b</sup>	0.003 <sup>c</sup>	0.002 <sup>a</sup>	6.820 <sup>b</sup>	13.031 <sup>b</sup>	0.013 <sup>b</sup>	0.691 <sup>b</sup>	0.032 <sup>b</sup>	0.097 <sup>b</sup>	0.138 <sup>a</sup>	0.019 <sup>a</sup>	0.0069 <sup>a</sup>
Arka Nikethan	1.469 <sup>c</sup>	0.042 <sup>c</sup>	0.017 <sup>a</sup>	0.001 <sup>b</sup>	4.913 <sup>c</sup>	9.160 <sup>c</sup>	0.016 <sup>b</sup>	0.543 <sup>b</sup>	0.031 <sup>b</sup>	0.056 <sup>c</sup>	0.114 <sup>b</sup>	0.017 <sup>a</sup>	0.0081 <sup>a</sup>

Values followed by a similar lower-case letter within treatments are not significantly different according to Fischer LSD test at  $P < 0.05$ .

## 4. Discussion

According to the "optimal foraging theory" the differences in the volume of nectar by each cultivar is a prime factor to the honey bee with respect to evolutionary energetics [19, 21, 16]. This theory assumes that the insects, generally behave in a manner that allows them to reduce the energy expended for foraging activity [7]. Thus, bees should prefer to visit the flowers that provide the greatest reward per visit. The energy

sugar concentration varies for different varieties too. Twelve different sugars viz., ribose, arabinose, xylose, fucose, glucose, fructose, galactose, mannose, inositol, sorbitol, sucrose and maltose were detected when nectar was analysed under LC-MS as detailed in Table 3. Among them, fructose, glucose, and sucrose were the major sugars with respect to pollinator preference. Nectar sugar composition of all the onion varieties was predominantly hexose (fructose and glucose) dominant with less sucrose concentration.

Nectar of Arka Pragathi had significantly higher level of fructose (20.339 mg/g) and glucose (10.99 mg/g) and much less sucrose (0.151 mg/g) respectively. Cultivar Arka Kalyan produced nectar with 13.031 mg/g of fructose, 6.820 mg/g of glucose and 0.138 mg/g of sucrose, which was significantly different from that of Arka Nikethan which produced less of these sugars. Nectars of all the tested cultivars characterised as hexose dominant since sucrose-hexose ratio [S/(G+F)] for Arka Pragathi (0.0048), Arka Kalyan (0.0069) and Arka Nikethan (0.0081) was found to be less than 0.1 (Table 3).

### 3.4 Relationship between bee foraging and nectar characteristics

The number of honey bee visits differed on different onion varieties. Foraging populations of *A. cerana*, *A. florea* and *T. iridipennis* mostly preferred varieties with a higher volume of nectar, higher sugar content and higher caloric value (Table 1&2). Arka Pragathi variety which produced a significantly higher volume of nectar (1.81  $\mu\text{l}$ /floret), TSS (63.80), energy (19.33 joules) attracted a large number of *A. cerana* (2.28), *A. florea* (4.14) and *T. iridipennis* (1.73). Arka Nikethan variety which produces significantly less nectar (1.37  $\mu\text{l}$ /floret), TSS (53.60) and Energy (12.29) attracted less number of *A. cerana* (1.58), *A. florea* (3.12), *T. iridipennis* (0.12) respectively. The *A. dorsata* population was naturally much lower in experimental site and showed no significant difference between different cultivars for different nectar parameters. A highly significant and positive correlation existed between foraging populations, nectar volume and TSS in all the honey bee species except *A. dorsata* for nectar volume.

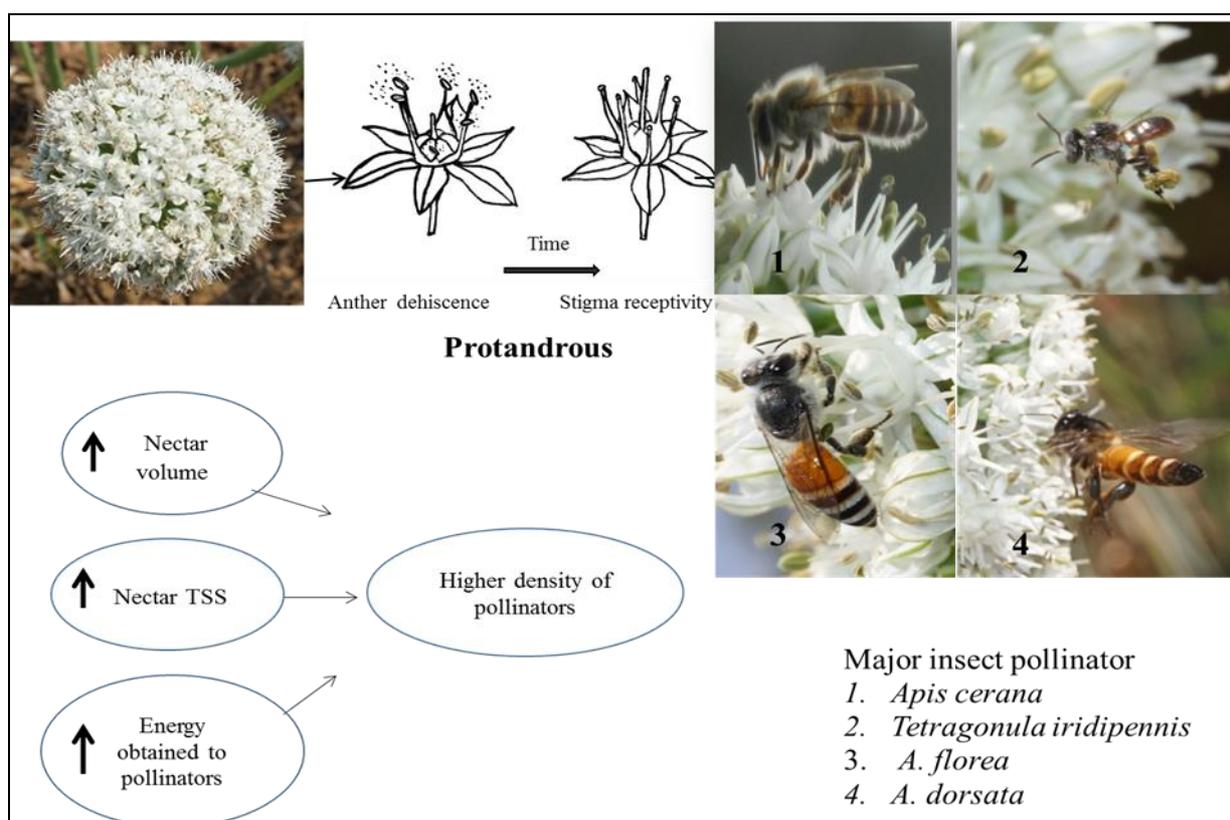
spent by the foragers and the caloric reward of the flowers must strike a balance if cross-pollination is to be maximized [12].

In our study onion flowers attracted a wide array of insects belonging to different Orders. Among them *A. florea* was found to be a dominant pollinator followed by *A. cerana*, *A. dorsata* and *T. iridipennis*. (Fig. 1) This result was supported by the findings of Sunitha *et al.* (2014) [23] who reported that

all the four *Apis* species were the key workers on onion umbel as pollen and nectar gatherers but *A. florea* was the abundant pollinator of onion compared to rest of the species [18]. Abrol (2010) [3] also found to be the *A. florea* as the most efficient forager of onion flowers. The reasons for the same, is quoted to be the capacity to work at high temperatures, maximum abundance, duration of activity period and foraging rates. While collecting nectar or pollen, *A. florea* pollinate large number of flowers by flying across the umbel. All the above studies showed similar results with our current results, making *A. florea* one of the top honey bee species in foraging activity of the onions.

Thus attractiveness of flowers of different cultivars was measured in terms of the mean number of foragers visited them during full blossom period. Arka Pragathi variety attracted the maximum number of pollinators followed by

Arka Kalyan and Arka Nikethan. Different plant species and their varietal difference for pollinator attractiveness have been studied by various authors. Abrol (2007) [2] observed that the foraging activity of honey bees differed on different cultivars of *Brassica*. Similarly, the Arizona onion varieties like Grano and Delta Giant attracted the most foraging honey bees among the six cultivars tested (Hagler *et al.* 1990) [11]. Varietal difference in attractiveness to different cultivars may be due to the volume of nectar produced by flowers and its sugar composition. In our study, the Arka Pragathi attracted a higher number of foragers as the volume of the nectar and its content was higher in its quantity as well as quality compared to the other varieties. This variation, in nectar volume and TSS among different varieties of same crop species, could be due to physiological or genetic factors [2].



**Fig 1:** Schematic diagram of onion flower, major pollinators, relationship between bee foraging and nectar parameters

Nectar secretion is essential to plants, which are dependent on insect pollination for reproduction. Onion flowers are a rich source of nectar. Onion flowers do not produce nectar immediately upon opening. The flowers of 4, 5 and 6 days old age were at nectar production stage. At this stage, maximum foragers get attracted to flowers. This result is in accordance with Hagler *et al.* (1990) [11] who worked on six onion cultivars and reported that the onion flowers bloomed over a six day period and produced nectar during the last three days. Nectar sugar analysis of all the onion varieties tested had hexose dominance with fructose and glucose being the major ones, which is an important indicator of pollinator preference. The short-tongued or lapping insects use nectar which is usually hexose-rich and of fairly high sugar concentration [14]. Fructose was found to be the predominant sugar in all the three onion varieties tested. Fructose content varied from 20.339 mg/g (Arka Pragathi) to 9.160 mg/g (Arka Nikethan) of nectar. Glucose is the second major sugar ranged from

10.999 mg/g (Arka Pragathi) to 4.913 mg/g (Arka Nikethan). Sucrose was very negligible among important sugars. Our result was supported by the findings of Silva and Dean (2000) [20] that onion nectar was predominantly hexose with undetectable levels of sucrose concentration of sugar in the nectar varied markedly between onion hybrid parents. Hagler *et al.* (1990) [11] confirmed that fructose was the dominant sugar, glucose was almost as abundant as fructose and sucrose was least abundant sugar in all the three onion cultivars studied.

There was a positive correlation between the number of bee foragers, nectar volume and TSS. As the nectar volume and TSS value increased, the number of honey bee visitation increase (Fig. 1). This was clearly confirmed by Abrol (2007) [2] that the *Brassica* cultivars with higher nectar concentration attracted a larger number of bees and there was a highly significant and positive correlation existed between foraging population (*A. cerana* and *A. mellifera*) and nectar volume,

sugar concentration and caloric value on different brassica cultivars. Corbet (1978) who found that the size of foraging populations is largely determined by the nectar quality and quantity. Results of Heinrich and Raven (1972) <sup>[12]</sup> supported our findings that honey bees tend to forage on those plants that give a greater caloric return for the energy expended in search and extraction of the nectar. The high quantity of nectar can increase number of visitor bees <sup>[20]</sup>.

## 5. Conclusion

In the present investigation, onion flowers attracted a wide range of pollinators, among them four honey bee species namely *Apis cerana*, *Apis florea*, *Apis dorsata* and *Tetragonula iridipennis* were found to be major pollinators. The *A. florea* was the most dominant as well as efficient pollinator of onion compared to other honey bee species. Our study revealed that higher is the floral reward, better is the foraging activity. Nectar being the primary reward for insect foragers, its quantity and quality had adequately influenced honey bee activity, which in turn affects pollination. Hence varieties with copious amount of nectar with the right proportion of sugars attract a higher number of honey bees thus results in optimum pollination. Arka Pragathi cultivar attracted significantly more number of honey bees. Further, the nectar secretion physiology, plant and flower phenology of these cultivars need to be studied for better understanding of the plant pollinator preference.

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