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Short communication

Overhead sprinkler irrigation affects pollinators and pollination in pumpkin (*Cucurbita maxima*)

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ABSTRACT

Cucurbits are monoecious plants and most of them depend on pollinators for fruit set. However, studies suggest that pumpkin and squash fields are pollination deficient. Pollinator limitation, suboptimal flower sex ratio, and exploitative competition between invasive ants and bees are a few major suggested reasons for this. Pumpkin flowers have a corolla cup and secrete nectar throughout anthesis, which make them attractive to bees. In south India, honey bee (*Apis cerana*) is the major pollinator of pumpkin flowers. Like pollinators, water is also a limiting factor for growth and production of flowers in pumpkin. It has been suggested to use overhead sprinklers to irrigate and fertilize pumpkin fields. We hypothesized that the sprinkler affects the visitation characteristics of foraging bees in flowers and pollination, and so, we performed an experiment to test this. For this reason, we recorded pollinator visits on staminate and pistillate flowers for 15 min and poured water in the flower cups and examined the water retention time and the visitation characteristics of pollinators in both staminate and pistillate flowers, and fruit set in pistillate flowers. Water remained inside the staminate and pistillate flower cups until the flower senescence. Honey bees entered and foraged nectar/pollen in flowers during the waterless condition. In water-filled flowers, honey bees hovered around or landed only on the corolla and did not forage floral resources or contact the reproductive parts. As a consequence, none of the water-filled flowers developed fruits. Therefore, the overhead sprinkler irrigation, despite cover a large area effectively, may not be an appropriate irrigation method for pumpkin during flowering period.

1. Introduction

The plant family Cucurbitaceae has a large number of edible crop plants in the genera, *Cucurbita, Cucumis, Luffa, Benincasa, Momordica,* and *Citrullus*. They are commonly called as cucurbits. Cucurbits are monoecious plants, *i.e.* they produce staminate (male) and pistillate (female) flowers on different axils, but, on same plants. They produce a large number of staminate flowers than pistillate flowers (Stapleton et al., 2000; Allesh et al., 2019). Anusree et al. (2015) found that over 98%, 90%, and 70% of the total flowers produced, respectively, on bitter-gourd, ash-gourd, and pumpkin, respectively, are staminate. Allesh et al. (2019) studied flowers of 325 plants of pumpkin (*Cucurbita maxima*) in two complete flowering seasons (2015–2016 and 2016–2017) and found that 31,656 flowers and 6,055 flowers produced were staminate and pistillate, respectively. Allesh et al. (2019) also suggested that high staminate/pistillate flower sex ratio is required for optimal pollination in pumpkin.

Most of the cucurbits are pollinator-dependent for fruit set (Petersen et al., 2013; Carr and Davidar, 2015). Global studies suggest that pumpkin is pollination deficient (Garibaldi et al., 2013), and the following reasons have been suggested for pollination deficiency in pumpkin: (1) lack of diversity (Petersen et al., 2013) or functional group diversity of pollinators (Hoehn et al., 2008), (2) poor pollinator-friendly management of field (Julier and Roulston, 2009), (3) competitive and interference competition from the invasive ants to the pollinators (Sinu et al., 2017), and (4) suboptimal staminate to pistillate flower sex ratio in field (Allesh et al., 2019).

Pumpkin is pollinated by honey bees, bumble bees, and squash bees in different parts of the world (Petersen et al., 2013), but, in south India, honey bee is the major pollinator of pumpkin flowers. The flowers are showy yellow and large with a cup formed by the fusion of five petals. This cup holds a cone shaped anther in staminate flowers and a three-lobed ringed stigma in pistillate flowers in the inner middle part of the flower. The nectar is accumulated at the basal part of the reproductive structures of the flowers in the flower cup. Both the staminate and pistillate flowers produce adequate amount of good quality nectar as a reward for the floral visitors (Ashworth and Galetto, 2001), and it is secreted continuously throughout the entire period of anthesis.

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The flowers receive the visits not only by the bees, but also by the ants (Sinu et al., 2017; Unni et al., under review). While most of the ant species forage nectar from flowers, some use it as a predation ground to prey upon the pollinators. Sinu et al. (2017) showed that the globally-invasive ant – *Anoplolepis gracilipes*– is a major flower visitor of pumpkin, which exerts both the interference and competitive pressures to honey bees, the major pollinator of pumpkin in Malabar region of Kerala (Sinu et al., 2017).

Like pollinators, water is also an indirect, but, an important limiting factor of pollination process in plants (Turner, 1993; Petandou et al., 1999; Carroll et al., 2001; Gillespie et al., 2015; Waser and Price, 2016; Gallagher and Campbell, 2017). Studies showed that the pollen and nectar production in flowers can vary with the soil moisture (Turner, 1993; Petandou et al., 1999; Carroll et al., 2001; Gillespie et al., 2015; Waser and Price, 2016). In *Ipomopsis aggregata*, the nectar production linearly increased with the moisture level in the soil (Waser and Price, 2016). In onion, the nectar production was high in the intermediate level of soil moisture (Gillespie et al., 2015).

Agricultural crop fields sometimes maintain a good population of pollinators, but, the poor management of field due to extensive use of insecticides and herbicides, tillage of soil, irrigation method, might directly or indirectly affect the pollinators' visits to the flowers and the pollination process (Mayer and Lunden, 2001; Julier and Roulston, 2009; Gillespie et al., 2015; Mendes Barbosa et al., 2019). It may damage the flowers, change the production pattern of pollen or nectar in the flowers, or interfere the chemical interaction between flowers and pollinators (Gillespie et al., 2015; Burkle and Runyon, 2016; Mendes Barbosa et al., 2019), and, consequently, leading to suboptimal crop production (Gillespie et al., 2015; Mendes Barbosa et al., 2019).

The vines and fruits of gourds, pumpkins, and melons are water rich. Therefore, water is critical for both the vegetative growth of the plants, reproduction, and fruit development. So, pumpkin plants need moist loamy soil with good drainage capacity for optimum growth of plants and production of flowers and fruits (Simonne et al., 2010; Kelley and Langston, 2014). In order to maintain the soil moisture during summer cultivation of cucurbits, it has been suggested to use overhead sprinklers, furrows, and drip irrigation methods (Simonne et al., 2010; Kelley and Langston, 2014).

The small-growers of south India traditionally use pot water to irrigate pumpkin plants. They pulley water and pour that directly in seed pits twice a day (personal observation). This is laborious, and might damage the vines if not walked carefully through the field. So, many growers have shifted to use a pump (1.5–2 inches diameter) or overhead sprinklers to irrigate the field vegetables that include the cucurbit crops as well. While the hose pump irrigation damaged the vines and the flowers due to the forceful discharge of water, the overhead sprinklers filled water inside the flower cups, so much so that the reproductive parts of both the staminate and pistillate flowers have been immersed in the water. The growers usually water plants between 06.30 a.m. and 08.00 a.m., which is coincided by the flower opening time of pumpkin flowers. Considering this practice, we hypothesized that this might affect the behavior and foraging characteristics of pollinators of pumpkin flowers. In the present study, we asked the following questions: A) Will water remain in pumpkin flowers? If so, how long will the water be remained in the flowers? B) Are the visitation frequency and the time spent by honey bee different between staminate and pistillate flowers in their water-less condition? C) Will water-logging affect visitation characteristics of pollinators in pumpkin flowers and fruit set in pistillate flowers?

2. Material and methods

This study took place in two sites (Aingoth (12°15.31′ N and 75°07.59′ E; 20 m a.s.l.) and Thavidisserry (12°18.339′ N and 75°15.914′ E; 139 m a.s.l)) of the Kasaragod district of the state of Kerala in south India during the November – April cultivation season of 2018–2019. The farmers use the paddy land to cultivate vegetables in the fallow period of paddy as an intercrop. The growers, based on the land holding size and the source of water, arrange 10–50 seed pits. In each pit, three seeds were sown. The trailing vines were allowed to spread on the ground. The growers manure the plants using cow dung, ash, and neem cake. Please see Sinu et al. (2017) for more agronomic practices the growers of the study area followed. The farms have loamy sandy soil that has a very short water retention capacity. Therefore, the growers irrigate the pumpkin plants twice a day.

We measured the quantity and the sugar concentration of the nectar secreted by both the staminate (N = 6) and pistillate flowers (N = 6) using 10 μl microcapillary tubes (Drummonds) and a hand-held refractometer (0–40%), respectively. The pre-anthesis buds were covered using a mosquito net bag a day before their opening. There was a great number of bee visits in the early period of anthesis. The bees – often more than one at a time – spent over five minutes per visit in pistillate flowers for harvesting nectar (Unni et al., under review), which suggests that the nectar secretion in flower might be continuous. In order to measure the quantity of nectar produced per five minutes, we harvested the nectar using microcapillary tubes three times in 5-min intervals for each selected flower immediately after the anthesis (around 06:00 to 07:00 a.m).

We scheduled a flower watch program to record the visitor species, the number of visits, and the time the visitors spent during each of their visits for foraging nectar/pollen from both the staminate and pistillate flowers. Altogether, we observed 31 pistillate and 40 staminate flowers in the two fields. We opened the pre-covered staminate and pistillate flowers immediately after the anthesis took place and observed them for 15 min, which normally occurred between 06:00 a.m. and 07:00 a.m. Then, we poured water inside the flower cups (Fig. 1) and observed the visitors for another 15 min between 07:00 a.m. and 07:45 a.m. Later, we tagged the flowers and observed them hourly until the time of senescence (around 02:00 p.m.) to find out the water retention time inside the flower cups. Then, we maintained the tag on pistillate flowers to study fruit set index.

In order to qualify a bee's visit in a waterless flower, the bee had to

Fig. 1. Water-held pistillate (A) and staminate (B) flower of *C. maxima*.

enter inside the flowers. In water-holding condition, if a bee had approached, lingered, and attempted to land on a flower or landed on a petal, we considered that as a visit. The number of times the bees entered (waterless) or approached (water-holding) the flower in 15 min was considered as the visitation frequency. The time a bee actually spent inside the flower cup for foraging nectar/pollen was recorded as the time spent by the bee.

We compared the visitation frequency and the time spent by the bees in waterless staminate and pisitllate flowers to study whether the bees' visitation characteristics were different between the flower sexes. We used a generalized linear model (GLM) with poisson error as the distribution type and **log**as a link function to study the effect of flower sex on the visitation frequency of the bees. We used a linear model (LM) with **log**y + 0.1 transformed time spent data to study the effect of flower sex on the time the bees spent on the flowers. In order to study the effect of water inside the flowers on the visitation characteristics of pollinators, we compared the visitation frequency and the time spent by honey bees in flowers during its waterless and water-holding conditions using a GLM and Wilcoxon test, respectively. We used the experiment type as fixed effect, poisson as the distribution type, and **log**as the link function in the GLM model.

3. Results and discussion

Immediately after the anthesis, a pistillate and a staminate flower produced an average of 5.2 μL (± 0.62 s.e.) and 2.9 μL (± 0.34 s.e.) nectar per five minutes, respectively. The difference in the amount of nectar secreted per five minutes in staminate and pistillate flowers was significant (Student's *t-test:* $t = 3.40$, $p = 0.009$). The sugar concentration of the nectar of a pistillate (30.5% ± 2.15 s.e.) and a staminate flower (29.6% ± 1.14 s.e.) was not significant ($t = 1.68$, $p = 0.13$).

Honey bees (*Apis cerana*) were the exclusive pollinators of pumpkin flowers during the study. Previous studies in the same study area also suggest that *A. cerana* is a major pollinator of pumpkin flowers (Allesh et al., 2019; Sinu et al., 2017). In this study, neither the visitation frequency (GLM: -0.14 ± 0.28, $z = -0.51$, $p = 0.61$) nor the time the bees spent inside the flowers (LM: -0.52 ± 0.67, $t = -0.77$, $p = 0.44$) were significantly different between pistillate and staminate flowers (Fig. 2).

The water, once filled inside the flower cup, was stored until the flowers were senesced. Because the flowers are large and showy, bees were attracted to the flowers regardless of the condition of the flowers. Therefore, the flowers during the water-filled and waterless conditions received similar numbers of visits of honey bees (GLM: 0.33 ± 0.22, $z = 1.47$, $p = 0.14$). This indicates that the bees were present in the fields during the observation period, but, found the water that is filled in the flowers, a difficulty to enter inside the flowers. However, the bees did not land on the reproductive parts of the water-filled flowers, despite a few of them landed on the corolla. Therefore, the time the bees spent inside the flowers during the water-filled (mean = 0 s.) and waterless conditions (mean = 14.3 ± 2.58 (s.e.) sec.) was significantly different ($W = 20$, $p < 0.00005$) (Fig. 3). All the pistillate flowers monitored in the present study had received multiple visits of honey bees before we poured water in the flower cups (Fig. 2), but, none of them developed into fruits. This suggests that the pollen-pistil interaction might also have been hampered by the water inside the flowers.

Pumpkin is an obligate cross-pollinated plant (Sinu et al., 2017) and bees are the major vectors of their pollen grains (Sinu et al., 2017; Petersen et al., 2013; Hoehn et al., 2008). Seven or more visits of pollinators to both the staminate and pistillate flowers are a minimum requirement to pollinate the flowers (citations in Julier and Roulston, 2009). Previous studies found that honey bee is a predominant pollinator of pumpkin flowers in the study area (Sinu et al., 2017; Allesh et al., 2019). In the present study too, honey bee was an exclusive pollinator of pumpkin flowers. Ants were present in some flowers. But, since they were not pollinators of the flowers (Sinu et al., 2017), we did not include them in the present analyses. Nevertheless, it is important to mention that the ants also did not enter inside the water-filled flowers.

Soil wetness increases soil biodiversity; therefore, the irrigated pumpkin fields contain more ground-nesting wild bee pollinators of pumpkin (Julier and Roulston, 2009). However, previous studies have suggested that the irrigation method, if inappropriate to the crop flowers, might negatively affect the flower and its specialist pollinators in the flowers (Gillespie et al., 2015; Burkle and Runyon, 2016; Mendes Barbosa et al., 2019). It might tear the petals or corolla tube of the flowers and directly affect the physical attractiveness of the flowers. It might indirectly affect the visitation characteristics of pollinators by manipulating the pollen display or nectar secretion pattern in the flowers. Also, it might affect the chemical interaction between the flowers and the pollinators.

In our study, the honey bees made similar number of visits to the waterless and water-filled pumpkin flowers in overhead sprinkler-irrigated fields, which suggests that the sprinkler irrigation did not reduce the attractiveness of the flowers to the bees. Like our study, Mendes Barbosa et al. (2019) found that the sprinkler irrigation had little

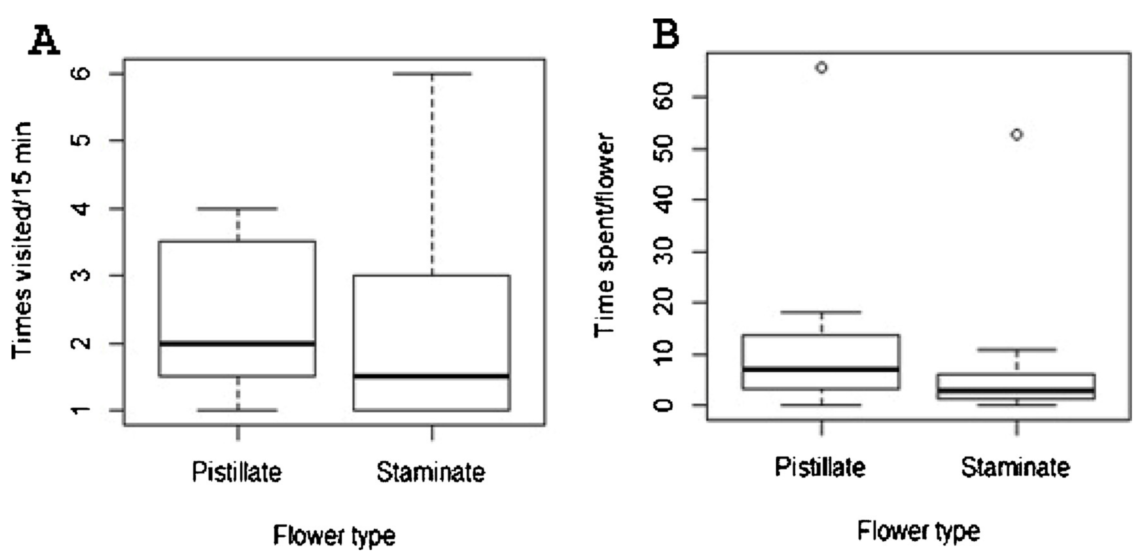

Fig. 2. Visitation frequency per fifteen minutes (A) and time (Seconds) honey bees spent per flower per visit (B) in pisitllate and staminate flowers of *Cucurbita maxima*.

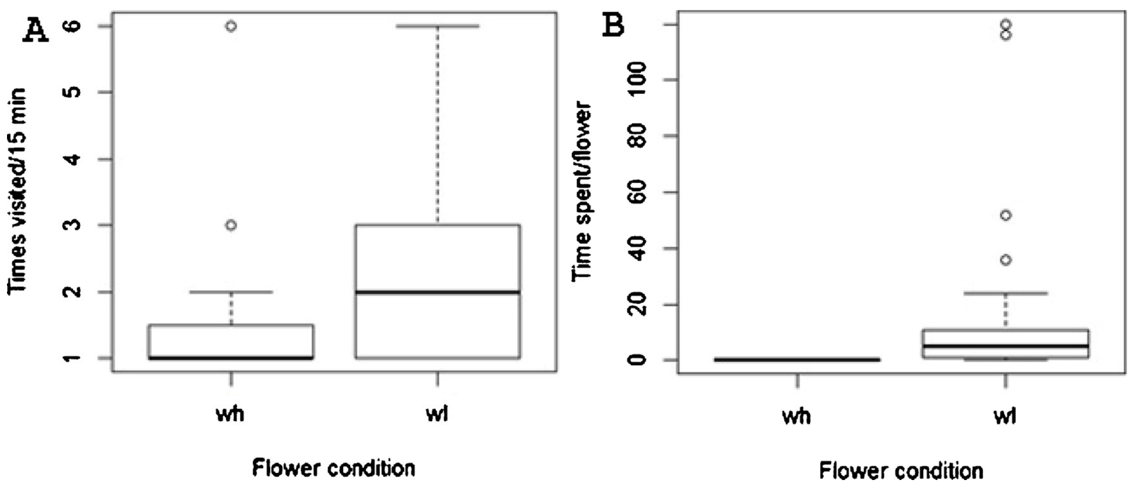

Fig. 3. Visitation frequency per fifteen minutes (A) and the time (Seconds) the honey bees spent per flower per visit in the flowers before and after the water was poured inside flower cups of pumpkin. The data on staminate and pistillate flowers were pooled before the analysis (wh = water-holding flower; wl = waterless flower).

impact on the visitation rate or species richness of pollinators in onion flowers. They also expected that the overhead sprinkler irrigation might facilitate pollination as the sprinkled large water droplets might give a mechanical vibration in the poricidal anthers of onion flowers (Mendes Barbosa et al., 2019). But, Mayer and Lunden (2001) had a different finding in their onion fields; they reported 94% reduction in honey bees' foraging visits in overhead sprinkler-assisted onion fields. It is, therefore, likely that the flower shape and flower architecture might suggest the direction of the impact of sprinkler irrigation on pollinators and pollination process in plants. Unlike the pendent flowers of onion, the pumpkin flowers are erect with a large flower cup. It stores water and immerses the reproductive parts in both the staminate and pistillate flowers. Therefore, the sprinkler irrigation disrupted pollination in pumpkin flowers not by affecting the visitation rate of pollinators, but, by making a physical barrier (water column) between the pollinators and the reproductive parts of the flowers.

Healthy population of pollinators is required for adequate visitation rate to the flowers. Simultaneously, the poor field management might interfere the pollinator visits to the flowers (Gillespie et al., 2015; Mendes Barbosa et al., 2019). Since pumpkin is a highly water-demanding plant, daily irrigation is necessary for maintaining good health of the plants, production of flowers, and development of fruits. Based on that, Kelley and Langston (2014) suggested that overhead sprinkler irrigation to be used to irrigate and fertilize pumpkin and squash plants. However, although the water-filled and waterless flowers received similar numbers of visits of honey bees, the overhead sprinkler irrigation may be avoided in pumpkin fields, at least during the flowering phase. We found that the flowers stored water until their senescence, which make impossible the presence of pollinators inside the flowers, and consequently, their contact to the reproductive parts of the flowers. None of the pistillate flowers, that had the visits of honey bees before the water was stored, developed fruits. Therefore, it is worth investigating whether the water inside the pollinated flowers affect the remaining process of pollen-pistil interaction.

In the United States – a major producer of pumpkin – both overhead sprinkler and drip irrigation are in use to irrigate pumpkin fields (Julier and Roulston, 2009; Kelley and Langston, 2014). Julier and Roulston (2009) found a positive effect of irrigation on nest abundance of ground-nesting bees in pumpkin fields. Their irrigated fields had either drip or overhead sprinkler for irrigating the plants, but, they have not studied which irrigation method make the pumpkin flowers pollinator friendly. We suggest that for large pumpkin fields, drip irrigation might be an efficient substitute of overhead sprinkler irrigation during the flowering phase of pumpkin. We expect that our study will generate interest among pollination biologists around the world to further test the efficiency of overhead sprinkler irrigation on other wild pollinators.

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