

Article

Rearing of Native Bumblebee Species *Bombus haemorrhoidalis* for Greenhouse Pollination in Pakistan[†]

Umer Ayyaz Aslam Sheikh^{1,*}, Munir Ahmad², Muhammad Asif Aziz², Muhammad Imran¹,
Junaid Rahim¹, T'ai Roulston³, Shengnan Guo⁴ and Cheng Sun^{5,*}

¹ Department of Entomology, University of Poonch Rawalakot, Azad Jammu and Kashmir, Rawalakot 12350, Pakistan

² Department of Entomology, Pir Mehr Ali Shah Arid Agriculture University, Murree Road, Rawalpindi 46000, Pakistan

³ Blandy Experimental Farm, College of Arts and Sciences, University of Virginia, 400 BLANDY FARM LN, Boyce, VA 22620, USA

⁴ Hengshui Center for Disease Prevention and Control, Hengshui 102206, China; hsgsn1989@126.com

⁵ College of Life Sciences, Capital Normal University, Beijing 100048, China

* Correspondence: umerayaz@upr.edu.pk (U.A.A.S.); cheng.sun@cnu.edu.cn (C.S.)

[†] This paper is part of the PhD thesis of Umer Ayyaz Aslam Sheikh presented to Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan.

Abstract: Greenhouse tomato production is a growing business worldwide, and it is strongly promoted by bumblebee pollination. Although there are over 250 bumblebee species worldwide, very few species have been reared successfully for greenhouse tomato pollination. Those successfully managed species, especially *Bombus terrestris*, are shipped around the world for commercial use. However, managed bumblebees are known to escape greenhouse facilities, have established local populations, spread disease to local bumblebees, and are blamed for the declines of some indigenous bee species. An alternative to shipping exotic bumblebees around the world is to develop local species for greenhouse pollination. Such an approach has the dual benefits of creating a new industry of insect rearing while reducing threats to local bee communities. In this study, we successfully reared *Bombus haemorrhoidalis*, which is the most common bumblebee species in Northern Pakistan, in a laboratory and compared its effectiveness as a tomato pollinator with that of commercial *B. terrestris* in a greenhouse. We found that the effectiveness of *B. haemorrhoidalis* in tomato pollination in a greenhouse is very similar to that of *B. terrestris* when it comes to the fruit size, number of seeds, and fruit weight. Our study provides an example of how to rear a native bumblebee species to pollinate local crops, which is a method that could potentially substitute the importation of non-indigenous bumblebees.

Keywords: bumblebee; *Bombus haemorrhoidalis*; greenhouse pollination; rear local species; tomato



Citation: Sheikh, U.A.A.; Ahmad, M.; Aziz, M.A.; Imran, M.; Rahim, J.; Roulston, T.; Guo, S.; Sun, C. Rearing of Native Bumblebee Species *Bombus haemorrhoidalis* for Greenhouse Pollination in Pakistan. *Agriculture* **2024**, *14*, 590. <https://doi.org/10.3390/agriculture14040590>

Academic Editor: Elena Gonella

Received: 11 August 2023

Revised: 13 March 2024

Accepted: 26 March 2024

Published: 8 April 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The greenhouse production of fruits and vegetables is an important and growing industry worldwide. Bumblebees are the most important pollinators of greenhouse fruits, especially tomato and pepper. Greenhouse tomato is the main crop that is pollinated by bumblebees, and the value of tomato crops is estimated to be about USD 12 billion per year [1]. Besides tomato, melon, watermelon, cucumber, cherry, sweet and hot pepper, apple, plum, and apricot are also economically important crops that are pollinated by bumblebees [2]. Tomatoes are among the most important crops due to their nutritional values and economic impact. Globally, China is the world leading producer of tomato, followed by Turkey, India, and the United States, while Pakistan has produced 12.6 million metric tons during the 2019 to 2020 agricultural period.

In the past, the pollination of tomatoes was carried out mechanically, which cost around EUR 1000 in 1988. Since then, the domestication of bumblebees and their use in

pollination has significantly reduced the cost of this process. Due to their several abilities, like buzz pollination, and their insulated bodies, they are ideal for efficient pollination, and other domestic or wild bees are less effective (Chandler et al., 2019 [3]). The pollination of crops by bumblebees could reduce the cost of mechanical/manual pollination and increase fruit yields and quality, leading to higher sales prices [1].

While there are over 250 bumblebee species worldwide [4], only 5 species (*Bombus terrestris*, *Bombus impatiens*, *Bombusignitus*, *Bombus lucorum*, and *Bombus occidentalis*) have been successfully reared and used in greenhouse pollination. *Bombus terrestris* is commercially reared throughout Europe and China, while *Bombus impatiens* is mainly reared in North America. Among these species, *B. terrestris* was the first artificially reared species to be used in greenhouse pollination, and now, it is the most widely used commercial species throughout the world. Bumblebees can now be reared in industries and used around the world [5]. The Netherlands, Israel, and Belgium are the main countries that export bumblebees, while China, Italy, Spain, Mexico, and Japan are the main countries that import bumblebees. It is estimated that two million bumblebee colonies are being reared by over 30 factories throughout the globe for glass and greenhouse crops as commercial pollinators in tomato, cucumber, bell pepper, strawberries, and many other vegetables and fruits [6].

The interaction between commercially producing bumblebee pollinators and flowers has been found in the spillover of several pathogens to other native wild bees, which is the cause of several diseases [7]. As the most producing bumblebee species of the world, *B. terrestris* was recently reported as a major risk factor for the transfer of pathogens and diseases from managed colonies to wild populations of this species and other bumblebee species around the world [7,8]. The use of non-indigenous species of bumblebees in greenhouse pollination poses risks to the local bumblebee fauna. The importation of bumblebees could cause the spread of parasites and pathogens from imported bumblebees to native bees, which will affect native flora and fauna in the long run [1,9]. Among these pathogens, *Carthadial bombi* is the most prevalent one, causing significant damage to the local fauna of bees. These imported parasites have been found to significantly damage native species of the area. Moreover, these imported bees were also found to compete with native bumblebee species by replacing them, and they have become the cause of bumblebee decline in their native regions [5]. Most of the pathogens and diseases are transferred from commercially producing bumblebees to wild bumblebees when reared bees escape from greenhouses and interact with the wild population in nature. To reduce the risk of transmitting parasites and pathogens to wild populations, the use of native bumblebee species for commercial crop pollination purposes is much appreciated and has been introduced in many regions [8].

Among the 250 bumblebee species, 13 bumblebee species were found in Northern Pakistan in different agriculture crops and wild flora [10]. However, the European bumblebee species *B. terrestris* is the primary pollinator of greenhouse crops in Pakistan. *Bombus haemorrhoidalis* is the most common and only *Bombus* species in the lower northern part of Pakistan, which pollinates both wild and cultivated crops in nature. *B. haemorrhoidalis* is present in the Margalla Hills, Muree Hills, and Himalayan Region of Pakistan [11]. About 43 wild and cultivated plants were recorded as foraging host plants and are only pollinated by this *Bombus* species. The goals of this study were to (1) rear the native Pakistan bumblebee species *B. haemorrhoidalis* from its wild queens; (2) maintain lab colonies across the years; and (3) compare its effectiveness as a greenhouse pollinator with that of *B. terrestris*. This study represents an endeavor to rear local bumblebee species for crop pollination to substitute the importation of non-indigenous species, which poses ecological risks. This study will be helpful to develop mass rearing technologies for this native bumblebee species and may introduce a new species to the pollination world. It will decrease the risk of transferring pathogens and diseases through imported bumblebees to non-native regions and also decrease the economic burden of the importation cost.

2. Materials and Methods

2.1. The Rearing of Native Bumblebee Species

In the early spring, *B. haemorrhoidalis* queens emerging from winter hibernation were collected from different locations of the Margalla and Muree hills of lower Northern Pakistan. All of the bumblebee queens were collected using entomological aerial nets during foraging activities on their host plants. Mostly queens were collected from wild flowers of *Silybum marianum*, *Adhatoda zeylanica*, *Robinia pseudoacacia*, *Malus domestica*, *Lantana camara*, *Saussurea* sp., *Impatiens* sp., and *Mentha longifolia* during early and mid-hours of the day.

Queens were kept in 50 mL falcon tubes with sugar solution, and two or three small holes were made in falcon tubes. Falcon tubes were placed in water coolers with ice pads to manage the temperature and humidity during travel from field to laboratory. For 24 h, captured bumblebee queens were shifted to dark in plastic boxes, and sugar solution was provided as nectar feeding. Each queen was shifted into a plastic starter box (15 × 20 × 8 cm³) lined with cardboard at the bottom. Two false bumblebee pupae made with honeybee wax were provided in the center of the box to encourage the queens to lay eggs.

Many small holes were produced in the upper lid of the box to avoid moisture amassing and proper ventilation. A small plastic container filled with 50% sugar solution and pollen pallet (made with sugar solution and grinded pollens) were kept in the box for the purpose of feeding the queens. Colony starter boxes were kept in a dark room with a low light intensity (10 lux) to decrease light disturbance during egg laying under controlled conditions at a temperature of 26–28 °C and relative humidity around 60–70%. Bumblebee queens were left in starter boxes for 7 days, and then boxes were opened to observe the queens' egg laying activities. Queens that laid eggs were separated from queens that did not lay eggs. After the emergence of the first full batch, the colony was shifted in large slanted BioBest bumblebee colony boxes. Fresh pollen grains in small Petri dishes and 50% sugar solution were continually provided as feed. Colonies were regularly checked for pest or predator presence and cleaned to avoid any disease or contamination. When the colony reached its mature stage (i.e., having more than 150 workers), *B. haemorrhoidalis* workers were collected for pollination efficiency evaluation for tomato plants in greenhouse.

2.2. Greenhouse Conditions

Pollination efficiency evaluation experiments were performed in one greenhouse (2000 m²), which is a part of a hydroponics farm (19,800 m²) and located at Pir Mehr Ali Shah Arid Agriculture University (Rawalpindi, Pakistan). The greenhouse has a glass–aluminum structure of Dutch technology and is equipped with a computerized drip irrigation system. Also, the greenhouse is covered with a Venlo insect screen. Inside the greenhouse, the relative humidity was maintained between 65 and 80%, and the average daily temperature was maintained between 18 and 26 °C; such relative humidity and temperature were maintained throughout the experiments. Tomato plants (Grandella cultivar) were grown in pots of rock wool (an area of 12 cm²), which were placed in cocoa peat slab (100 cm in length and 23 cm in width) 40 cm above the ground; the density of tomato plants was 2.5 plants per square meter. Experiments were performed in mid-September when tomato plants had been maintained for five months following standard commercial procedures.

2.3. The Evaluation of the Pollination Efficiency

For the bumblebee pollination experiment, two bumblebee species, *B. haemorrhoidalis* and *B. terrestris*, were compared for their pollination efficacy on greenhouse tomatoes. Colonies of *B. haemorrhoidalis* were reared locally in a lab (Department of Entomology, Pir Mehr Ali Shah Arid Agriculture University) as described above, while *B. terrestris* colonies were imported from BioBest company (Belgium). It was ensured that there was no presence of any pests or predators in the imported bumblebee colonies, and these colonies

had a founder queen with good health. For each bumblebee species, the colony with 130–150 foraging workers with a healthy founder queen was selected for the pollination experiment and transferred from the lab to glasshouse for pollination experiment. Four bee boxes for each species were placed in four different sections of the same glass house; in each section, tomato plants were enclosed with poly film, and one bee box was placed inside. In each beehive box, there was a plastic container situated at the base, serving as a reservoir for a sugar solution. This solution acted as a substitute for nectar to support the nutritional needs and overall health of the bumblebees, especially since tomato flowers do not naturally produce nectar. After 48 h, twenty trusses that had marks indicating a bumblebee visit (i.e., the presence of brown, bruised spots on anther cones) were randomly selected from twenty different plants for each bumblebee species' pollination experiment, and those trusses were then covered with muslin cloth bags until the fruit characteristics were measured.

For manual pollination and self-pollination experiments, twenty newly developed trusses from twenty different plants were randomly selected for each pollination experiment, respectively, which were then covered with muslin cloth bags for the avoidance of bumblebee disturbance. Manual pollination experiment was performed on selected trusses for one week, once per day, using a mechanical vibrator. Self-pollination experiment was carried out by leaving the selected trusses untreated.

For all of the above pollination experiments, when the tomato fruits were matured (fruit color became orange-red or darker [12]), they were harvested and frozen for further measurements of the fruit characteristics.

2.4. *The Statistics of the Flower Visitation Time and Foraging Activity (Flower Visitation Time)*

To estimate the flower visitation time for each bumblebee species, the time spent on a single flower, which starts from the bumblebee's landing on the flower, followed by its working process to the time it leaves, was measured (in seconds) by using a stop watch. A total of one hundred randomly selected individuals were used to estimate the flower visitation time for each species.

The foraging activity of each species' colony was observed in the first, third, and seventh week after the placement of a bee box inside the greenhouse, and incoming and outgoing bumblebee workers were counted and recorded for 5 min at three time points (8–9 am, 11–12 pm, and 5–6 pm), respectively. Five such observations were performed for each time point and for each species, with their means being compared statistically using the LSD test at 5% probability. Linear regression was employed to differentiate between the time spent and the foraging activity of both species.

Microl Origin 7.0 software was used to graphically show the visitation rate of bumblebee foragers.

2.5. *Qualitative and Quantitative Parameters of Tomato Fruits*

After collecting tomato fruits from selected trusses as per the treatments, the parameters of fruit size and quality were measured. The diameter and height of tomato fruits were measured in millimeters by using a Vernier caliper, and the weight of the fruit was measured in grams via electronic balance. Roundness index was calculated by dividing the maximum height of each tomato by its minimum. Each fruit was thawed, and most of its pulp was removed; the remaining pulp was pushed through a sieve (mesh size 20), and the remaining seeds were counted as described [12].

Prior to analysis, data were subjected to the normality test using the Shapiro–Wilk test. The means of the number of seeds, weight, height, and diameter and roundness index of the tomato fruits were compared statistically using ANOVA Least Significant Difference test ($p < 0.05$).

3. Results

3.1. The Successful Rearing of the Native Bumblebee Species *Bombus haemorrhoidalis*

The rearing conditions we used (see Methods) are pretty similar to the rearing technologies of *B. terrestris* [1]. In total, we collected sixty-three (63) *B. haemorrhoidalis* queens from wild flora, out of which thirty-nine (39) could lay eggs, and thirty-six (36) of them reached mature stage with healthy bumblebee workers (Figure 1). In the mature colonies, the number of workers ranged from 260 to 320; the number of males varied from 110 to 135; and the number of daughter queens ranged from 21 to 48. Our study indicates that wild *B. haemorrhoidalis* could be artificially reared in a lab and its commercial production can be carried out on a large scale with a relatively high success rate, and it can become an alternate European bumblebee species for this region.



Figure 1. One well-developed colony of *B. haemorrhoidalis*.

3.2. Pollination Efficiency of *B. haemorrhoidalis* and *B. terrestris*

We evaluated the pollination efficiency of *B. haemorrhoidalis* on greenhouse tomatoes and compared its efficiency with that of other methods. Our results indicated that, while manual pollination was better than self-pollination, bumblebee pollination was significantly better than manual pollination when it came to tomato production (heavier and larger fruits with more seeds; Table 1). In addition, *B. haemorrhoidalis* was as good a pollinator as *B. terrestris* in all measures of tomato production (Table 1). *B. haemorrhoidalis* produced a significant difference in the tomato yield and tomato quality than the manual and self-pollination methods.

The maximum fruit weight was recorded for the tomatoes that were pollinated by *B. terrestris* (116.9 ± 2.01 g/fruit), followed by the tomatoes pollinated by indigenous *B. haemorrhoidalis* (111.0 ± 2.05 g/fruit) (Table 1). The tomatoes pollinated by *B. haemorrhoidalis* were found with the highest number of seeds/fruits, the largest fruit height, and the largest fruit diameter (132.5 ± 2.29 , 55.36 ± 0.43 and 58.73 ± 0.37 , respectively), followed by the tomatoes pollinated by *B. terrestris* (129.3 ± 2.02 , 54.3 ± 0.46 and 57.33 ± 0.36). The self-pollinated tomatoes were found with the lowest fruit weight, seed numbers per fruit, height of fruit, and fruit diameter (16.76 ± 2.33 , 12.8 ± 2.15 , 16.93 ± 1.75 , and 19.7 ± 1.95 , respectively). For the self-pollinated tomatoes, the fruit weight, seed number, and fruit

height were seven times, ten times, and three times smaller than those of the tomatoes pollinated by bumblebees, respectively. The largest fruit roundness measure (3.92 ± 0.094) was observed for the self-pollinated tomatoes, while the smallest fruit roundness (1.14 ± 0.11) was observed for the tomatoes pollinated by *B. terrestris* (Table 1).

Table 1. Influence of different pollination methods on qualitative and quantitative characteristics of tomatoes.

Characters	Self-Pollination	Manual Pollination	<i>B. haemorrhoidalis</i> Pollination	<i>B. terrestris</i> Pollination	<i>p</i> -Value
Weight (g)	16.76 ± 2.33 c	79.677 ± 9.12 b	111.0 ± 2.05 a	116.9 ± 2.01 a	<0.001
Seed (number)	12.8 ± 2.15 c	88.59 ± 5.41 b	132.5 ± 2.29 a	129.3 ± 2.02 a	<0.001
Height (mm)	16.93 ± 1.75 c	43.66 ± 1.86 b	55.36 ± 0.43 a	54.3 ± 0.46 a	<0.001
Diameter (mm)	19.7 ± 1.95 c	47.68 ± 2.09 b	58.73 ± 0.37 a	57.33 ± 0.36 a	<0.001
Roundness	3.92 ± 0.094 a	2.21 ± 0.44 b	1.18 ± 0.11 c	1.14 ± 0.11 c	<0.001

The letters in the table indicate the significant differences. The values show the mean ± SE, and if they are followed by a different letter, they are significantly different at $p < 0.05$ based on the Least Significant Difference Test.

Taken together, our results indicate that tomato plants pollinated by bumblebees exhibit better characteristics that are directly related to the tomato yield and quality than those of manual and self-pollinated tomato plants, and *B. haemorrhoidalis* could serve as an alternate pollinator of tomato plants in greenhouse conditions.

3.3. Foraging Activities of *B. haemorrhoidalis* and *B. terrestris* on Tomato Flowers

For both bumblebee species, the maximum number of workers was observed coming back to their hives from their tomato flower visitation in the first week during the morning (08:00–09:00 am), with the minimum incoming number being observed in the early morning of the seventh week (05:00–06:00 pm). In the fourth week after the placement of bee colonies inside the greenhouse, the incoming bee traffic decreased compared to that of the first week, which decreased to a negligible level in the seventh week (Table 2).

Table 2. Trafficking of incoming bumblebees to hives after tomato visitation.

Weeks	<i>B. haemorrhoidalis</i>			<i>B. terrestris</i>		
	08:00–09:00 am	11:00–12:00 am	05:00–06:00 pm	08:00–09:00 am	11:00–12:00 am	05:00–06:00 pm
1st	4.75 a	3.25 bc	1.500 de	5.50 a	3.50 b	1.50 cd
4th	4.25 ab	3.25 bc	1.00 e	3.25 b	2.25 bc	0.05 ef
7th	1.50 cd	1.00 e	0.25 e	1.25 de	0.75 ef	0.0

Values followed by different letter indicate significant difference.

The outgoing workers of both bumblebee species were at their maximums in the first week during the morning (08:00–09:00 am), while the minimum was observed in the seventh week during the evening (05:00–06:00 pm). The decrease in outgoing workers from their hives was observed in the fourth week, which was less than the first week but should be sufficient for tomato pollination; the outward movements are negligible in the seventh week for both species (Table 3).

Table 3. Tracking of outgoing bumblebees from hives for tomato visitation.

Weeks	<i>B. haemorrhoidalis</i>			<i>B. terrestris</i>		
	08:00–09:00 am	11:00–12:00 am	05:00–06:00 pm	08:00–09:00 am	11:00–12:00 am	05:00–06:00 pm
1st	6.00 a	3.00 b	1.00 cd	6.00 a	2.75 bc	0.50 de
4th	5.50 a	2.25 bc	0.25 d	4.25 b	2.00 cd	1.00 de
7th	1.00 bc	0.75 cd	0.25 d	1.25 cd	0.75 de	0.00 e

Values followed by different letter indicate significant difference.

3.4. *B. haemorrhoidalis* Spends Slightly More Time on Tomato Flower Visitation than *B. terrestris*

The time that each species spent on a single tomato flower during foraging was measured. The variation in visitation time between *B. terrestris* ($Y = 0.029x + 2.452$; $R^2 = 0.199$) and *B. haemorrhoidalis* ($Y = 0.028x + 2.623$; $R^2 = 0.190$) was investigated by a linear regression analysis at first (Figure 2A for *B. terrestris* and Figure 2B for *B. haemorrhoidalis*). The time spent by *B. haemorrhoidalis* on each flower ranged from 1 to 8.7 s, while *B. terrestris* (Figure 2) spent 1.02 to 8.63 s. On average, the time *B. haemorrhoidalis* spent on one flower in one visit was 4.03 ± 0.19 s, which is slightly longer than that of *B. terrestris* (3.93 ± 0.19 s) (Figure 3).

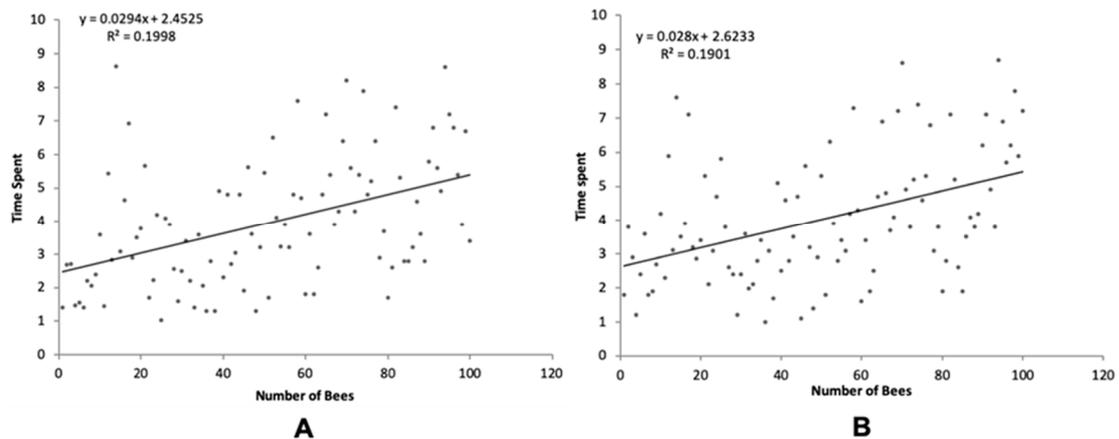


Figure 2. Linear regression analysis indicating time spent by *B. terrestris* (A) and *B. haemorrhoidalis* (B) on tomato flowers during foraging (in seconds).

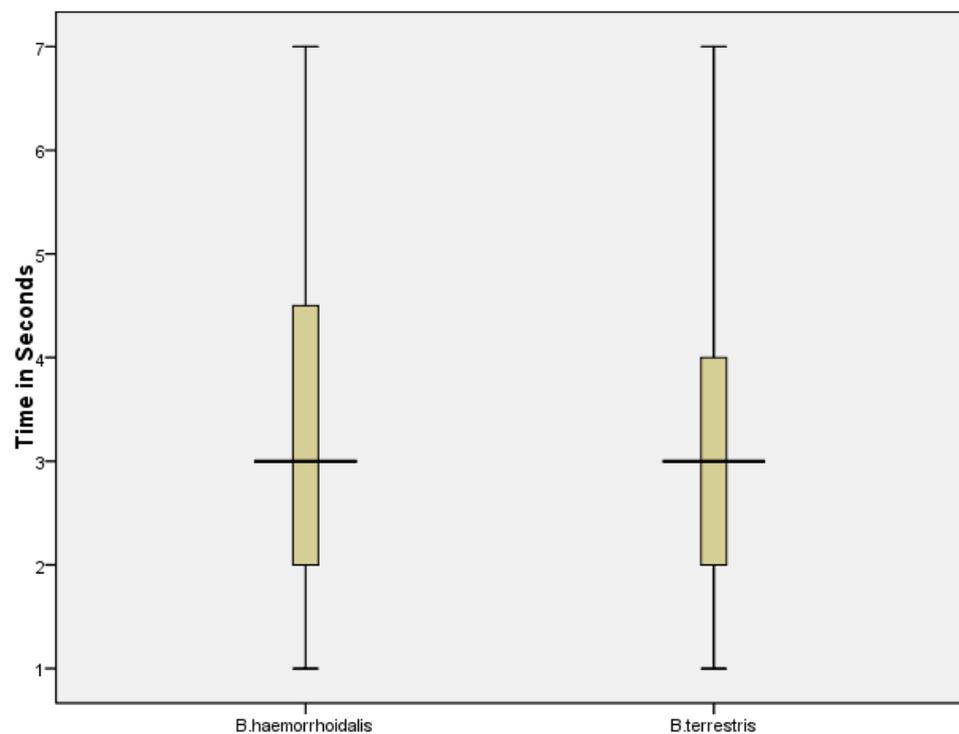


Figure 3. The average time spent on one tomato flower in one visit (in seconds) by *B. haemorrhoidalis* and *B. terrestris* during their foraging activities.

4. Discussion

In this study, we reared *B. haemorrhoidalis* colonies from their wild queens and used *B. haemorrhoidalis* as tomato pollinators under a hydroponic crop system in a glasshouse condition for the first time. Our results suggest that wild *B. haemorrhoidalis* could be reared into mature colonies with hundreds of workers with a high success rate, and the pollination of greenhouse tomato by *B. haemorrhoidalis* produced similar result (tomato production) as when *B. terrestris* were used (Table 1), indicating that *B. haemorrhoidalis* could be used as an alternative pollinator to *B. terrestris* for greenhouse tomato pollination.

According to the results of our study, both *B. terrestris* and *B. haemorrhoidalis* workers preferred to forage flowers during the morning, and their activity decreased through the midday to evening (Tables 2 and 3). The decrease in foraging activity in the midday could be related to the increased temperature and light intensity of the greenhouse. The foraging activities of both bumblebee species decreased as the time passed and decreased to a negligible level after the placement of bee colonies into a greenhouse for four weeks (Tables 2 and 3); foraging activities depend on the number of workers, the development stage of one colony, and the amount of food available inside the colony. With the passage of time, as the colony cycle moves to reproductive stages, the number of workers starts decreasing, and the foraging activities of the bumblebee colony decreases compared to its early stages [13]; the change in bumblebee foraging activity uncovered in this study could be due to the decreased number of workers inside the colony (or the completion of their life cycle). In this study, we found that *B. haemorrhoidalis* workers spent slightly more time on tomato flowers than *B. terrestris* (Figure 3), which might be related to their different body sizes, because *B. haemorrhoidalis* has a significantly larger size than *B. terrestris*.

Various techniques, for example, manual vibrators, the use of a plant growth regulator, and honeybee and bumblebee pollination, have been used to increase the production of tomatoes and other Solanaceae crops [14]. Compared with manual vibration and the use of a plant growth regulator in tomato crops, a higher yield and seed number were observed for bumblebee-pollinated tomatoes [15], which is consistent with the results obtained from our present study, and another more recent study reported the same findings regarding the increases in fruit size and seed numbers using commercially produced bumblebee pollinators in greenhouse conditions [16]. Also, bumblebee represents a better pollinator than honeybee under greenhouse conditions. Honeybees are not very effective pollinators in greenhouse or glasshouse conditions and also do not pollinate tomato crops in the same way as bumblebee species [17] because bumblebees can maintain their flying activity and pollinate flowers under cool conditions [18]. Thus, several bumblebee species, including *B. terrestris*, *B. ignitus*, *B. ephippiatus*, and *B. impatiens* are reared in laboratories and have been used to pollinate greenhouse plants; among them, *B. terrestris* is the most widely used bumblebee species and has been shipped around the world for greenhouse pollination. *B. impatiens* is used as a pollinator in North America and Canada, *B. ignites* is used in Korea, while *B. ephippiatus* is used in Mexico for greenhouse tomato pollination [1,19]. However, the importation of bumblebee colonies for local pollination purposes could lead to the importation of parasites and spread into local regions [9], which can infect native bee species. Similar findings of an increasing tomato yield and quality were obtained by the pollination of the local bumblebee species of China, *B. lantschouensis*, and this species showed the greatest potential in solar greenhouse conditions [20,21].

Various factors have been pinpointed as causes of bumblebee decline, such as habitat loss/degradation, heightened pesticide usage, and a rise in pathogens. Of particular concern to conservationists is the role of pathogens, especially those originating from commercial colonies, which are seen as a pressing issue in this decline [7,22].

The utilization of non-native bumblebees is leading to the transmission of pathogens and diseases among wild bumblebee populations. Bumblebees that are commercially bred are introducing pathogens to wild populations through interactions with them [7,23]. Occasionally, these bred bumblebees escape from greenhouses, come into contact with native wild populations, and transmit pathogens to them [3]. Moreover, the prevalence

of diseases in commercial hives may facilitate the invasion of pathogens into wild *Bombus* populations. *Crithidia bombi* is identified as the most frequently transferred pathogen from bred bumblebees to wild ones [24].

Recently, it has been noticed that the commercially reared *Bombus terrestris* is a risk factor for wild bumblebees due to the transfer of diseases and pathogens when it interacts with wild populations of bumblebees, and it also spreads pathogens to other wild bees [22,25,26]. Hence, it is essential to use specific bumblebee species that are suitable for commercial crop pollination services based on regional suitability [27]. It is crucial to recognize the potential risks to indigenous pollinators and manage them within the framework of both national and international commercial trade in bumblebees [26,28,29] because invasive parasites are the major reason for the decline of native bumblebees and other wild bees throughout the world [30].

The transfer of diseases and pathogens from commercially reared bumblebees for crop pollination is an alarming occurrence. To decrease the risk of pathogens being spread, there is an urgent need to develop mass rearing technologies for native bumblebees and to use these native bumblebees as greenhouse pollinators in respective local regions. This will reduce the disease and pathogen transfer and will also help to decrease the importation cost as an eco-friendly practice. Our current study represents an endeavor to rear and employ indigenous species for greenhouse pollination, and our results indicate that the native bumblebee species *B. haemorrhoidalis* could potentially substitute the importation of the non-indigenous *B. terrestris*.

5. Conclusions

The native bumblebee species *B. haemorrhoidalis* was equally efficient as the imported European bumblebee species *B. terrestris*. In greenhouse tomato pollination, the native species gave similar results regarding the fruit weight, fruit size, seed number, and fruit diameter. In our study, native bumblebee species play the same role as the imported ones. These results provide experimental proof that native bumblebee species can be raised and domesticated to be used in greenhouse or glasshouse tomato pollination as an alternative to commercially produced species. Further studies on the rearing of other native bumblebee species in different regions are needed; in the meantime, non-native species can be replaced by native bumblebee species.

Author Contributions: Conceptualization, U.A.A.S. and M.A.; methodology, U.A.A.S., M.A.A. and M.I.; software, J.R. and U.A.A.S.; validation, U.A.A.S. and T.R.; formal analysis, M.A.; investigation U.A.A.S., M.I., M.A. and M.A.A.; resources, U.A.A.S. and M.A.; data curation, U.A.A.S. and M.A.; writing—original draft preparation, U.A.A.S., T.R., S.G. and C.S.; writing—review and editing, C.S., S.G., U.A.A.S. and T.R.; supervision, U.A.A.S., M.A. and M.A.A.; project administration, U.A.A.S. and M.A.; funding acquisition, U.A.A.S., M.A. and C.S. All authors have read and agreed to the published version of the manuscript. We'd like to express gratitude to the National Natural Science Foundation of China (Grant No. 32270445), the Higher Education Commission of Pakistan under the Indigenous PhD Scholarship.

Funding: This research was funded by the Central Public-interest Scientific Institution Basal Research Fund (grant numbers Y2019XK13 and Y2021XK16).

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

Acknowledgments: We gratefully acknowledge the HEC, Pakistan and Non-Apis Bees Laboratory of Entomology Department, PMAS-Arid Agriculture University Rawalpindi, Pakistan.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Velthuis, H.H.; Van Doorn, A. A century of advances in bumblebee domestication and the economic and environmental aspects of its commercialization for pollination. *Apidologie* **2006**, *37*, 421–451. [[CrossRef](#)]
2. Willmer, P.G.; Bataw, A.A.M.; Hughes, J.P. The superiority of bumblebees to honeybees as pollinators: Insect visits to raspberry flowers. *Ecol. Entomol.* **1994**, *19*, 271–284. [[CrossRef](#)]
3. Chandler, D.; Cooper, E.; Prince, G. Are there risks to wild European bumble bees from using commercial stocks of domesticated *Bombus terrestris* for crop pollination? *J. Apic. Res.* **2019**, *58*, 665–681. [[CrossRef](#)]
4. Cameron, S.A.; Hines, H.M.; Williams, P.H. A comprehensive phylogeny of the bumblebees (*Bombus*). *Biol. J. Linn. Soc.* **2007**, *91*, 161–188. [[CrossRef](#)]
5. Winter, K.; Adams, L.; Thorp, R.; Inouye, D.; Day, L.; Ascher, J.; Buchmann, S. Importation of non-native bumblebees into North America: Potential consequences of using *Bombus terrestris* and other non-native bumblebees for greenhouse crop pollination in Canada, Mexico, and the United States. 2006. Available online: https://www.pollinator.org/pollinator.org/assets/generalFiles/BEEIMPORTATION_AUG2006.pdf (accessed on 25 March 2024).
6. Mah, Y.I.; Lee, M.Y.; Bilinski, M. Some Characteristics of Korean Indigenous Bumblebee Species (*Hymenoptera*; *Bombus* Spp.) under Laboratory Conditions. *VIII Int. Symp. Pollinat.-Pollinat. Integr. Crops Nativ. Plant Syst.* **2000**, *561*, 287–291. [[CrossRef](#)]
7. Otterstatter, M.C.; Thomson, J.D. Does Pathogen Spillover from Commercially Reared Bumble Bees Threaten Wild Pollinators? *PLoS ONE* **2008**, *3*, e2771. [[CrossRef](#)]
8. Saini, M.S.; Raina, R.H.; Khan, Z.H. Species diversity of bumblebees (*Hymenoptera: Apidae*) from different mountain regions of Kashmir Himalayas. *J. Sci. Res.* **2012**, *4*, 263. [[CrossRef](#)]
9. Goka, K.; Okabe, K.; Yoneda, M. Worldwide migration of parasitic mites as a result of bumblebee commercialization. *Popul. Ecol.* **2006**, *48*, 285–291. [[CrossRef](#)]
10. Sabir, A.M.; Suhail, A.; Ahmed, S.; Khalid, S. Diversity of bumblebees (*Bombini, Apidae: Hymenoptera*) in Northern Pakistan. *Int. J. Agric. Biol.* **2011**, *13*, 159–166.
11. Sheikh, U.A.A.; Ahmad, M.; Imran, M.; Nasir, M.; Saeed, S.; Bodlah, I. Distribution of bumblebee, *Bombus haemorrhoidalis* Smith, and its association with flora in lower Northern Pakistan. *Pak. J. Zool.* **2014**, *46*, 1045–1051.
12. Dogterom, M.H.; Matteoni, J.A.; Plowright, R.C. Pollination of greenhouse tomatoes by the North American *Bombus vosnesenskii* (*Hymenoptera: Apidae*). *J. Econ. Entomol.* **1998**, *91*, 71–75. [[CrossRef](#)]
13. Kwon, Y.J.; Saeed, S. Effect of temperature on the foraging activity of *Bombus terrestris* L. (*Hymenoptera: Apidae*) on greenhouse hot pepper (*Capsicum annuum* L.). *Appl. Entomol. Zool.* **2003**, *38*, 275–280. [[CrossRef](#)]
14. Sun, S.G.; Huang, S.Q.; Guo, Y.H. Pollinator shift to managed honeybees enhances reproductive output in a bumblebee-pollinated plant. *Plant Syst. Evol.* **2013**, *299*, 139–150. [[CrossRef](#)]
15. Morandin, L.A.; Laverty, T.M.; Kevan, P.G. Bumble bee (*Hymenoptera: Apidae*) activity and pollination levels in commercial tomato greenhouses. *J. Econ. Entomol.* **2001**, *94*, 462–467. [[CrossRef](#)] [[PubMed](#)]
16. Ozols, N.; Gailis, J.; Jakobija, I.; Jaško, J.; Zagorska, V. Bumblebee pollination activity in a commercial tomato greenhouse during the winter season. *Rural. Sustain. Res.* **2022**, *48*, 45–53. [[CrossRef](#)]
17. Corbet, S.A. Role of pollinators in species preservation, conservation, ecosystem stability and genetic diversity. *VII Int. Symp. Pollinat.* **1996**, *437*, 219–230. [[CrossRef](#)]
18. Dramstad, W.E.; Fry, G.L.; Schaffer, M.J. Bumblebee foraging is closer really better? *Agric. Ecosyst. Environ.* **2003**, *95*, 349–357. [[CrossRef](#)]
19. Vergara, C.H.; Fonseca-Buendía, P. Pollination of greenhouse tomatoes by the Mexican bumblebee *Bombus ephippiatus* (*Hymenoptera: Apidae*). *J. Pollinat. Ecol.* **2012**, *7*, 27–30. [[CrossRef](#)]
20. Zhang, H.; Zhou, Z.; Huang, J.; Yuan, X.; Ding, G.; An, J. Queen traits and colony size of four bumblebee species of China. *Insects Soc.* **2018**, *65*, 537–547. [[CrossRef](#)]
21. Zhang, H.; Zhou, Z.; An, J. Pollen release dynamics and daily patterns of pollen-collecting activity of honeybee *Apis mellifera* and bumblebee *Bombus lantschouensis* in solar greenhouse. *Insects* **2019**, *10*, 216. [[CrossRef](#)]
22. Whiteman, L.B. Bumble Bee Pathogen Prevalence Determined by Host Species. Master's Thesis, The Ohio State University, Columbus, OH, USA, 2023.
23. Sasso, R.; Iodice, L.; Woodcock, C.M.; Pickett, J.A.; Guerrieri, E. Electrophysiological and behavioural responses of *Aphidius ervi* (*Hymenoptera: Braconidae*) to tomato plant volatiles. *Chemoecology* **2009**, *19*, 195–201. [[CrossRef](#)]
24. Figueroa, L.; Sadd, B.; Tripodi, A.; Strange, J.; Colla, S.; Adams, L.; Duennes, M.; Evans, E.; Lehmann, D.; Moylett, H.; et al. Endosymbionts that threaten commercially raised and wild bumble bees (*Bombus* spp.). *J. Pollinat. Ecol.* **2023**, *33*, 14–36. [[CrossRef](#)]
25. Rowe, G.; Hagadorn, M.A.; Lindsay, T.T.T.; Malfi, R.; Williams, N.M.; Strange, J.P. Production of bumblebees (*Hymenoptera: Apidae*) for pollination and research. In *Mass Production of Beneficial Organisms*; Academic Press: Cambridge, MA, USA, 2023; pp. 559–579.
26. Eakins, J.; Lynch, M.; Carolan, J.C.; Rowan, N.J. Studies on the novel effects of electron beam treated pollen on colony reproductive output in commercially-reared bumblebees (*Bombus terrestris*) for mass pollination applications. *Sci. Total Environ.* **2023**, *899*, 165614. [[CrossRef](#)] [[PubMed](#)]
27. Christman, M.E.; Spears, L.R.; Koch, J.B.; Lindsay, T.T.T.; Strange, J.P.; Barnes, C.L.; Ramirez, R.A. Captive rearing success and critical thermal maxima of *Bombus griseocollis* (*Hymenoptera: Apidae*): A candidate for commercialization? *J. Insect Sci.* **2022**, *22*, 2. [[CrossRef](#)] [[PubMed](#)]

28. Evans, E.; Strange, J.; Sadd, B.; Tripodi, A.; Figueroa, L.; Adams, L.; Colla, S.; Duennes, M.; Lehmann, D.; Moylett, H.; et al. Parasites, parasitoids, and hive products that are potentially deleterious to wild and commercially raised bumble bees (*Bombus* spp.) in North America. *J. Pollinat. Ecol.* **2023**, *33*, 37–53. [[CrossRef](#)]
29. Lohrmann, J.; Cecchetto, N.R.; Aizen, N.; Arbetman, M.P.; Zattara, E.E. When bio is not green: The impacts of bumblebee translocation and invasion on native ecosystems. *CABI Rev.* **2022**. [[CrossRef](#)]
30. Meeus, I.; Brown, M.J.; De Graaf, D.C.; Smagghe, G.U.Y. Effects of invasive parasites on bumble bee declines. *Conserv. Biol.* **2011**, *25*, 662–671. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.