Pollination efficiency of stingless bee, *Tetragonula iridipennis* (Smith) on greenhouse cucumber, *Cucumis sativus* (Linnaeus)

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The present study deals with the foraging activities and pollination efficiency of *Tetragonula iridipennis* in cucumber (*Cucumis sativus*) under protected conditions. Experiments were conducted during two seasons (summer and winter) with three different treatments: open pollination (OP), pollinator exclusion (PE) and stingless bee pollination (SBP) in Assam, North East India. The foraging activity of *T. iridipennis* was checked in SBP treatment at different timings after installing a 15,504 cm^3 beehive. The pollination efficiency of *T. iridipennis* was also examined among the three selected treatments. The peak period of visitation on cucumber flowers was recorded during 0800-0900 h of the day during both seasons. The yield increase of SBP over PE during both seasons was five times higher. These findings indicate the significance of *T. iridipennis* as an effective pollinator in greenhouse cucumbers.

Keywords: Cucumber, foraging activity, pollination efficiency, stingless bee, yield.

PROTECTED cultivation or growing crops under greenhouse conditions is becoming popular, especially with off-season vegetables. Protected farming thus creates a physical barrier that inhibits natural pollinators from accessing flowers[1]. Cucumber is produced in the greenhouse, where bees are restricted for better control of plant development and climatic conditions. To deal with the constraints in protected farming, most farmers resort to human labour to assist in manual cross-pollination of the flowers, which increases the cost of cultivation and the time taken to pollinate the flowers[2]. Therefore, introducing natural pollinators that can adapt to the constraints of restricted habitats and satisfy the pollination requirements of crops under these conditions would be a more cost-effective option[3].

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Cucumber is a monoecious plant. Pollination is one of the most important aspects of optimal cucumber fruit production[4]. Pollinating insects are essential for the efficient pollination of monoecious Cucurbitaceae, such as cucumber, resulting in increased yield and quality[5].

Stingless bees, *Tetragonula iridipennis* (Smith), might be a lucrative choice to facilitate pollination in field and greenhouse crops because of their generalist eating habits and floral consistency. Protecting and maintaining stingless bees for pollination is becoming increasingly important in India and worldwide. They are effective pollinators of Compositae, Cruciferae, Malvaceae, Nuciferae and other economically important crops. They pollinate various crop types, being efficient pollinators of 18 crops and assisting in pollinating over 60 cultivated plant species[6]. The large variety of stingless bee species permits the selection of the most suitable one for a crop or crop system, as well as their maintenance and management for trade[7]. In some tropical regions, it has also been observed that stingless bees play an important role as pollinating agents of some native plants[8]. Stingless bees like *Hypotrigona gribodoi*, *Melipona bocandei*, *Melipona lendliana* and *Plebeina hildebrandti* were reported to be efficient pollinators of sweet melon[9]; they may be used in greenhouse crops as well. Furthermore, few researchers have reported the potential of stingless bees as pollinators in crops under protected conditions. Honeybees are not always the most efficient pollinators due to a variety of variables, including body size and flower size mismatches, limited nectar production, and specific pollen release mechanisms in some plants[10]. When honeybees fail to pollinate a crop efficiently, it is probably more cost-effective to consider a better pollinator–plant match.

A perusal of the literature reveals that less information is available regarding the pollination of stingless bees on cucumbers under protected conditions in Assam, North East India. Keeping in view the importance of stingless bees in pollination, we examined the efficiency of pollination of stingless bee, *T. iridipennis* in greenhouse cucumber (*Cucumis sativus* (Linnaeus)).

Materials and methods

Study area

The experiments were conducted during the summer (2020) and winter (2020–21) to determine pollination efficiency of *T. iridipennis* in cucumbers under protected conditions. Summer and winter seasons were chosen because farmers grow their fruits in these two seasons, mostly in and around the study area. Farmers also profit more during these seasons as cucumber is grown as an off-season crop under greenhouse conditions. The study was conducted in the Horticulture Experimental Farm, Department of Horticulture (26°43′N and 92°12′E), and experimental field, All India Coordinated Research Project (AICRP) on Honeybees and Pollinators, Department of Entomology (26°72′N and 94°19′E), Assam Agricultural University, Jorhat. NE India.

Experimental design

The experiments were conducted under two environments (open and greenhouse) with three treatments: open pollination (OP), stingless bee pollination (SBP) and pollinator exclusion (PE), with 30 replicates under each treatment. (Here, one plant denotes one replicate.) Thus, there were a total of 90 plants under observation. The SBP treatment was laid under greenhouse conditions in the Horticulture Experimental Farm. The OP and PE treatments were laid in the experimental field under AICRP on Honeybees and Pollinators. For PE treatment, the replicates were covered with customized nets of size 2 mm to restrict the entry of pollinators prior to flowering. A total of 70 sq. m area was covered under each treatment during both seasons. No pesticides were applied in all the three treatments.

Crop selection

A local monoecious cultivar was selected for cucumber cultivation and sown during both seasons for each treatment. Summer sowing of the crop was done in April 2020, and winter sowing in November 2020, under both open and protected conditions. The duration of the crop was 90–100 days. Various intercultural operations, viz. enriching the soil with vermicompost, irrigation and weeding, were done at timely intervals during the crop growth period. The application of pesticides was strictly avoided.

Setting up the beehive in the greenhouse

A 15,504 cm^3 (19 × 14 × 17 cm^3) wooden box comprising approximately 700–1000 stingless bees maintained when 20% to 25% of cucumbers were in flowering (after 50 days of sowing) under SBP treatment during both seasons and removed just before the harvesting period[11]. One week after setting up the beehive, when all the plants bore flowers (almost 100%), the bees had acclimatized to the new environment and started foraging on cucumber flowers; also, no bee mortality was observed.

Foraging behaviour

Observations on foraging behaviour were made during the flowering period of cucumber from morning to evening five times (0600–0700, 0800–0900, 1000–1100, 1500–1600 and 1600–1700 h) in a day. Observations were made during midday (1100–1500 h) because bees cannot forage under high temperatures. The foraging parameters observed were the number of stingless bee visits per flower, time spent by stingless bees per flower (sec) and pollen load (mg). The number of stingless bee visits per flower was observed at a time interval of 1 min by randomly selecting one flower from each replicate for 10 successive days in each season. So, total of 300 min time (1 h for each time duration) were observed per day for checking the foraging behaviour of stingless bee. The time spent by a bee was recorded on 30 flowers observed on each replicate in 10 days with the help of a stopwatch, following the methodology of Raj and Rana[12]. The observations on pollen load per trip were recorded at each time intervals for 10 days during the flowering period of cucumber, following the methodology of Erickson[13]. At first, 10 incoming adult bees from the SBP plot were caught using a hand sweep net. Pollen was removed from their body as well as corbicula with the help of a camel hair brush, collected on a glass slide, and weighed on an electronic weighing balance. Ten adults were selected for measuring the pollen load because the weight of pollen from one stingless bee is negligible.

Yield parameters

Different yield parameters, viz. fruit set (%), average fruit length (cm), fruit girth (cm), fruit weight (g) and yield per plant (kg) were observed from each replicate under three treatments. Subsequently, yield per hectare (tonne) was estimated after harvesting fruits in the experimental plots in both seasons. The percentage of fruit set was calculated using the formula

$$\text{Fruit set (\%)} = \frac{\text{Number of fruits produced per plant}}{\text{Number of female flowers per plant}} \times 100.$$

Data analysis

Analysis of variance (ANOVA) was used to compare foraging activities during summer and winter with a subsequent

Table 1. Foraging behaviour of *Tetragonula iridipennis* on cucumber during summer (2020) and winter (2020–21)

Time of observation (h)	No. of bees/flower/min (N = 30)		Time spent/flower/bee (sec; N = 30)		Pollen load per trip/10 bees (mg; N = 30)	
	Summer	Winter	Summer	Winter	Summer	Winter
0600–0700	0.73 ± 0.09	0.72 ± 0.09	9.38 ± 0.88	10.56 ± 0.95	5.11 ± 0.91	5.02 ± 1.24
0800–0900	1.06 ± 0.15	1.21 ± 0.16	11.57 ± 0.37	12.07 ± 0.99	6.50 ± 0.67	7.15 ± 0.39
1000–1100	0.76 ± 0.14	0.85 ± 0.07	8.17 ± 1.06	8.83 ± 0.94	5.30 ± 0.77	4.76 ± 0.65
1500–1600	0.57 ± 0.10	0.63 ± 0.15	7.30 ± 0.53	8.32 ± 0.71	3.32 ± 0.33	4.09 ± 1.03
1600–1700	0.51 ± 0.11	0.49 ± 0.08	7.15 ± 0.83	8.54 ± 0.70	2.93 ± 0.56	2.98 ± 0.45
Mean ± SD	$0.73^a \pm 0.21$	$0.78^a \pm 0.27$	$8.71^b \pm 1.83$	$9.67^b \pm 1.61$	$4.63^c \pm 1.48$	$4.80^c \pm 1.53$
F-value (1, 8)	0.12		0.76		0.03	
P-value (<0.05)	0.73		0.40		0.86	

Means within columns separated by ANOVA test at $P < 0.05$. Means in columns followed by the same letters shown in superscript are non-significantly different.

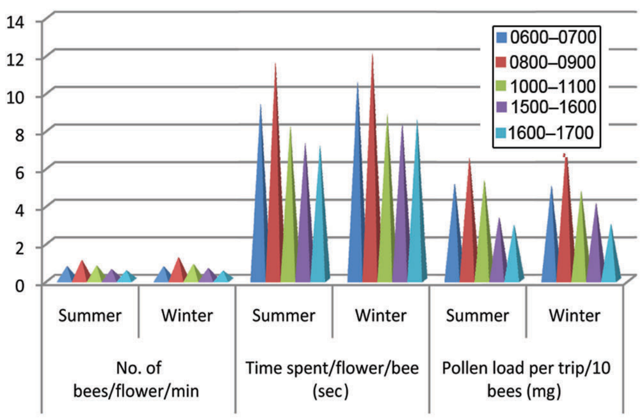

Figure 1. Foraging behaviour of *Tetragonula iridipennis* on cucumber during summer (2020) and winter (2020–21).

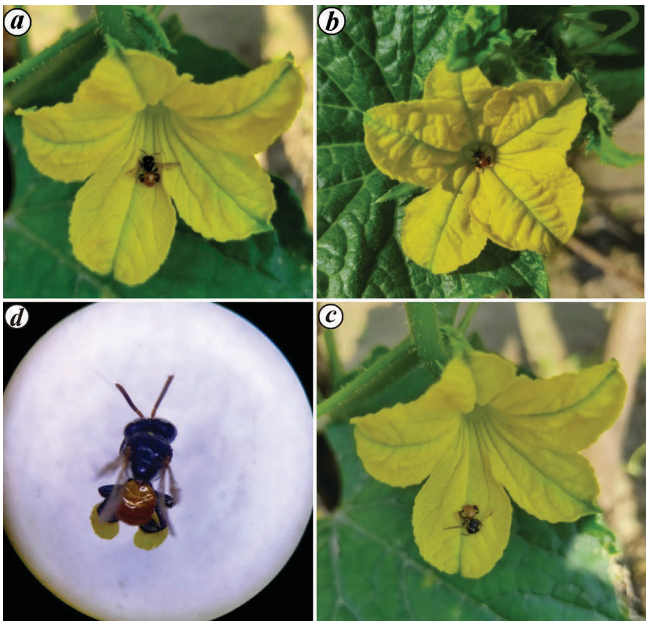

Figure 2. Foraging pattern of *T. iridipennis* on cucumber flower. ***a***, Initiation of foraging by the bee. ***b***, Active foraging period. ***c***, Departure of bee with corbiculate legs after completion of pollination. ***d***, Microscopic view of a worker bee with pollen load.

multiple comparison. Tukey test ($P < 0.05$) was performed to compare the yield attributes under different treatments (SBP, PE and OP)[14].

Results and discussion

Foraging behaviour of T. iridipennis on cucumber

The foraging activity of stingless bees on cucumber were meticulously checked during both seasons in the SBP treatment. Observations revealed that the peak foraging time of *T. iridipennis* on cucumber flowers was 0800–0900 h during both seasons. Studies have recorded the peak foraging activity of *T. iridipennis* during 0800–1100 h in Tamil Nadu[15] and Karnataka[16]. Two similar studies on the foraging activity of different stingless bees (*Melipona quadrifasciata* and *T. miniangkabau*) on tomato[17] and strawberry[18] plants respectively, revealed that the bees mostly visited the flowers of those plants during 0800–1100 h under greenhouse conditions. The mean number of *T. iridipennis* workers per flower per minute was 0.73 ± 0.21 during summer and 0.78 ± 0.27 during winter. Also, the highest number of *T. iridipennis* was recorded during 0800–0900 h in summer (1.06 ± 0.15) and winter (1.21 ± 0.16). The maximum number of *T. iridipennis*/m^2/10 min was observed to be 11 during 0900–1000 h in the morning and a minimum of 2 at 1700–1800 h (ref. 19), which also supports the present study. The average time required by *T. iridipennis* for foraging per flower was recorded higher during winter (9.67 ± 1.61 sec) than in summer (8.71 ± 1.83 sec). The present finding related to the average time spent by stingless bee which was recorded higher in summer and winter, highly contradicted with another previous finding where it was recorded the lower average time spent by stingless bee (3.9 ± 0.5 sec in TNAU orchard and 3.5 ± 0.3 sec in Srivilliputhur)[19]. Observations on pollen load revealed that during summer, the average pollen load carried by 10 *T. iridipennis* bees was 4.63 ± 1.48 mg/trip, and during winter 4.80 ± 1.53 mg/trip (Table 1 and Figure 1). The results slightly differ from those of Nicodemo *et al.*[20] who observed

Table 2. Effect of *T. iridipennis* pollination on cucumber yield during summer and winter, 2020–21

Treatment (N = 30)	Average fruit length (cm)	Average fruit girth (cm)	Average fruit weight (g)	Average fruit set (%)	Average fruit length (cm)	Average fruit girth (cm)	Average fruit weight (g)	Average fruit set (%)
	Summer (mean ± SD)				Winter (mean ± SD)			
Stingless bee pollination (SBP)	18.08^a ± 1.23	10.08^a ± 0.59	202.62^a ± 12.98	79.06^a ± 8.60	18.31^a ± 1.24	10.14^a ± 0.56	205.11^a ± 8.85	83.85^a ± 6.16
Pollinator exclusion (PE)	11.24^b ± 0.79	7.78^b ± 0.53	92.05^b ± 6.81	34.14^b ± 9.40	10.71^b ± 0.86	7.70^b ± 0.45	90.38^b ± 5.99	35.80^b ± 8.04
Open pollination (OP)	14.65^c ± 0.94	8.13^c ± 0.47	145.77^c ± 7.73	66.03^c ± 12.69	14.78^c ± 0.93	8.25^c ± 0.58	147.12^c ± 9.72	67.75^c ± 8.01
CD (0.05%)	0.88	0.47	8.34	9.05	0.89	0.46	7.27	6.50
F-value (2, 87)	345.63	160.56	1001.57	147.64	417.20	175.25	1420.00	323.54
P-value (<0.05)	4.03E-42	6.30E-30	8.73E-61	1.08E-28	2.60E-45	3.06E-31	3.79E-67	5.11E-41

Means within columns separated by Tukey's test at $P < 0.05$. Means in columns followed by the different letters shown in superscript are significantly different.

Table 3. Yield of cucumber obtained under different treatments during summer and winter, 2020–21

Treatment (N = 30)	Yield/plant (kg)	Yield/ha (tonne)	Yield increase over PE (%)	Yield increase over OP (%)	Yield/plant (kg)	Yield/ha (tonne)	Yield increase over PE (%)	Yield increase over OP (%)
	Summer (mean ± SD)				Winter (mean ± SD)			
Stingless bee pollination (SBP)	2.95^a ± 0.66	13.15^a ± 6.68	517.37	58.62	3.30^a ± 0.52	14.65^a ± 2.31	512.97	70.34
Pollinator exclusion (PE)	0.48^b ± 0.15	2.13^b ± 0.68			0.54^b ± 0.15	2.39^b ± 0.67		
Open pollination (OP)	1.86^c ± 0.36	8.29^c ± 1.60	289.20		1.93^c ± 0.30	8.60^c ± 1.33	259.83	
CD (0.05%)	0.39	3.47			0.31	1.38		
F-value (2, 87)	229.88	388.56			446.00	445.94		
P-value (<0.05)	1.88E-35	4.24E-44			1.86E-46	1.87E-46		

Means within columns separated by Tukey's test at $P < 0.05$. Means in columns followed by different letters in the superscript are significantly different.

that the average pollen load per stingless bee was 0.6 mg/trip, i.e. 6 mg/10 bees per trip in Africa. The differences in weather conditions of the experimental plots might have contributed to these variations. Statistical comparison of the three above-mentioned foraging parameters indicated a non-significant difference between summer and winter (Table 1). Figure 2 depicts the foraging patterns of *T. iridipennis*.

Effect of T. iridipennis pollination on cucumber

During summer and winter, there were significant differences in fruit length, girth, weight and fruit set of cucumber (Table 2). During summer, the average fruit length of cucumber was observed to be significantly higher in the SBP plot (18.08 ± 1.23 cm) followed by OP (14.65 ± 0.94 cm) and PE (11.24 ± 0.79 cm) plots (Table 2). Similarly, the highest fruit girth and fruit weight were recorded in the SBP plot, followed by OP and PE plots (Table 2). The percentage of fruit setting was also significantly higher in SBP (79.06 ± 8.60), followed by OP (66.03 ± 12.69), and lowest in PE (34.14 ± 9.40) (Table 2). A similar trend was also observed during the winter (2020–21). The fruit length, fruit girth and fruit weight were observed to be comparatively higher in SBP plot, followed by OP and PE plots. The highest percentage of fruit setting was also observed higher in the SBP plot (83.85 ± 6.16) followed by OP (67.75 ± 8.01) and PE (35.80 ± 8.04) plots (Table 2).

Significant differences in yield per plant and yield per hectare were evident among all three treatments (Table 3). During both seasons, the highest yield was observed in SBP treatment, followed by OP and PE treatments. Accordingly, yield per hectare showed significant differences among all the treatments. Figure 3 shows a comparison of the yield of these three treatments. The yield increase of SBP over PE during the summer was 512.37%, meaning that almost five times higher yield was produced in SBP plot compared to PE plot. In the case of OP, there was almost 58.62% more yield of cucumber in SBP plot compared to OP plot. During winter, similar results were recorded for yield increases of SBP over PE and OP (Table 3). The results are in conformity with those of Kishan *et al.*[16], who observed significantly higher values of yield attributes in SBP plot when compared to control plot in two different experimental locations (Coimbatore and Srivilliputhur, Tamil Nadu, south India). A study has also reported that pollination by Brazilian native stingless bees contributes to a significant increase in fruit diameter and length of cucumber compared to honeybees[20]. The findings also suggest that cucumber flowers pollinated by bees have better quality in terms of weight, length and diameter. Cucumber flowers pollinated by stingless bees may increase the quality of

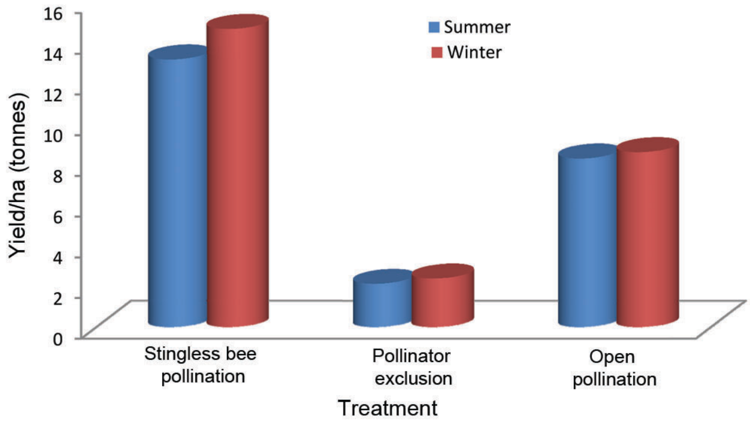

Figure 3. Yield of cucumber obtained under different treatments (tonne/ha) during summer (2020) and winter (2020–21).

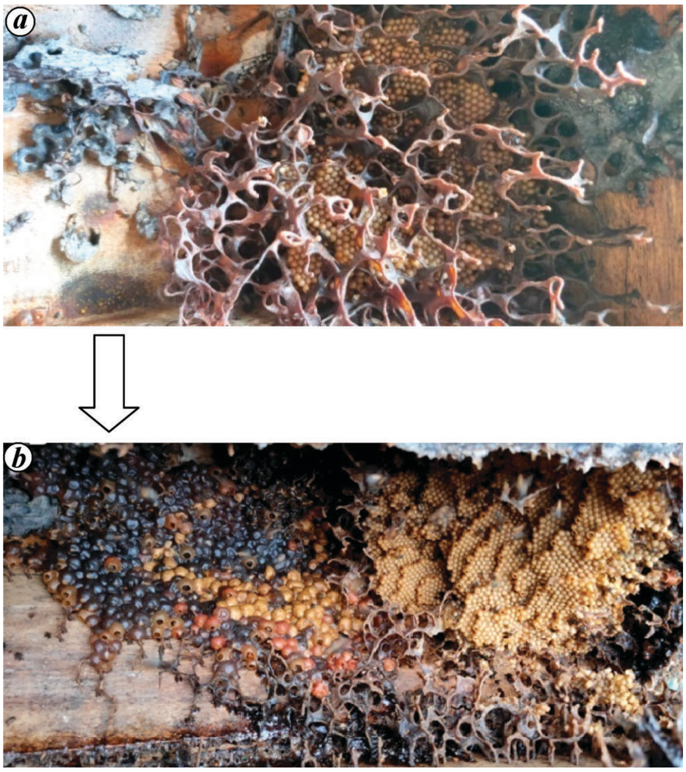

Figure 4. Pictorial evidences of same bee hive in two different conditions. ***a***, Almost empty hive before setting up in the greenhouse. ***b***, Hive filled with pollen, honey and brood area after stingless bee pollination.

fruit production[21]. dos Santos *et al.*[22], while studying the effectiveness of stingless bees *Scaptotrigona* aff. *depilis* and *Nannotrigona testaceicornis* as pollinators of cucumber plants in greenhouses reported that the highest cucumber yield was observed in those greenhouses that housed the stingless bees as pollinators during the Brazilian winter season. The results of experiments conducted in Malaysia also showed that the cucumber plants pollinated by stingless bees (*Heterotrigona itama*) produced heavier, longer and larger fruits than those produced from pollination without stingless bees[2]. A study on the pollination efficiency of *T. iridipennis* in watermelon reported larger fruits and greater yield in the case of bee-pollinated crops compared to control plot[23]. The enormous yield increase of cherry tomatoes under protected conditions using three native bees, i.e. *Melipona bicolor*, *Nannotrigona testaceicornis* and *Partamona helleri* has been reported[24], thus proving that they are effective pollinators under protected conditions. The bees visited the flowers frequently and carried maximum pollen load in the corbicula (pollen basket) present on their legs. However, in the PE plot where insect pollinators were restricted from visiting the flowers, limited pollination occurred by means of self- and/or wind pollination, resulting in improper pollination of flowers. This also resulted in flower dropping, poor fruit setting and lower yield. In the OP plot, as the plants were grown under open environmental conditions, an infestation of insect pests was observed, which resulted in a lower yield than the SBP plot. Figure 4 reveals that stingless bees act as effective pollen collectors, as observed from the transformation of pollen pots in the hive, which they collected from cucumber flowers during the flowering periods in pollinated plots.

The present study reveals that *T. iridipennis* is active as a pollinator in cucumber flowers under protected conditions during both summer and winter seasons because the bees swiftly adjust to confinement, maintain high population numbers and display suitable foraging activity. As a result, *T. iridipennis*-pollinated cucumber plot produced five times more yield than the control plot. A single wooden hive box (15,504 cm^3) of *T. iridipennis* can effectively pollinate cucumber flowers of a 70 sq. m area under protected conditions. Thus, we conclude that stingless bees (*T. iridipennis*) can be a good alternative as pollinators of cucumber flowers under greenhouse conditions where the use of other honey bees or pollinators could be restricted. Hence, cucumber plants can be successfully grown as off-season fruits with the help of stingless bees with regard to pollination efficiency.

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