

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

3 Sourav Sen^{1*}, Shimantini Borkataki², Pranjit Sutradhar³, Ritu Ranjan Taye⁴,
4 Badal Bhattacharyya², P. S. Saranya⁵, S. P. Nanda^{6,7} and M. Devender Reddy⁶

5 ¹Department of Agricultural Entomology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur,
6 Nadia-741252, West Bengal, India

7 ²Department of Entomology, Assam Agricultural University, Jorhat-785013, Assam, India

8 ³Department of Agronomy, Assam Agricultural University, Jorhat-785013, Assam, India

9 ⁴Regional Agricultural Research Station, AAU, Karimganj-788710, India

10 ⁵Polytechnic of Agriculture, ANGRAU, Garikapadu, Andhra Pradesh-521175, India

11 ⁶M. S. Swaminathan School of Agriculture, CUTM, Paralakhemundi-761211, Odisha, India

12 ⁷Department of Chemistry, School of Applied Sciences, CUTM, Paralakhemundi, Odisha –
13 761211, India

14 *For correspondence (email: souravsen561@gmail.com)

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25 **ABSTRACT**

26 The present investigation was undertaken to study the foraging activities and pollination
27 efficiency of *Tetragonula iridipennis* in cucumber under protected condition. The experiments
28 were conducted in two seasons (summer and winter) with three different treatments: open
29 pollination (OP), pollinator exclusion (PE) and stingless bee pollination (SBP) in Assam, India.
30 Foraging activity of *T. iridipennis* was checked in SBP treatment in different timings after
31 installing 15504 cubic centimeter hive. Pollination efficiency of *T. iridipennis* was also examined
32 among the selected 3 treatments. Peak period of visitation on cucumber flowers was recorded
33 during 0800-0900 hours of the day in both the seasons. The yield increase of SBP over PE in
34 both the seasons was found to be five times higher. These findings claim the significance of *T.*
35 *iridipennis* as an effective pollinator in greenhouse cucumber.

36 **Keywords:** Cucumber, foraging activity, pollination efficiency, stingless bee, yield

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46 INTRODUCTION

47 The protected cultivation or growing crops under greenhouse conditions is becoming
48 very popular, especially with off-season vegetables.¹ Protected farming thus creates a physical
49 barrier that inhibits natural pollinators from accessing flowers. Cucumbers are produced in
50 greenhouses where bees are kept out for better control of plant development and climatic
51 conditions.² To deal with the issues, most farmers resort to human labor to assist in manual
52 cross-pollination of the flowers, which increases cost of cultivation and the time it takes to
53 pollinate the flowers.³ As a result, introducing natural pollinators that can adapt to the constraints
54 of restricted habitats and satisfy the pollination requirements of crops under these conditions
55 would be a more cost-effective option.

56 Cucumber is monoecious plant.⁴ The pollination service is one of the most important
57 aspects of optimal cucumber fruit production.⁵ Pollinating insects are essential for the efficient
58 pollination of monoecious Cucurbitaceae, such as cucumber, resulting in increased yield and
59 quality.

60 Stingless bees, *T. iridipennis* might be a lucrative choice to facilitate pollination in field
61 and greenhouse crops because of their generalist eating habits and floral consistency. The
62 protection and maintenance of stingless bees for pollination purposes is becoming increasingly
63 important in India and throughout the world. They are effective pollinators of Compositae,
64 Cruciferae, Malvaceae, Nuciferae and other economically important crops.⁶ They pollinate a
65 variety of crop types and known as efficient pollinators of 18 crops and assist to pollination of
66 over 60 cultivated plant species.⁷ The large variety of stingless bee species permits the selection
67 of the most suitable species for a crop or crop system as well as their maintenance and
68 management for trade.⁸ In some tropical regions, it has also been observed that stingless bees

69 play an important role as pollinating agents of some native plants.⁹ Another finding showed that
70 stingless bees like *Hypotrigena gribodoi*, *Melipona bocandei*, *Melipona lendliana*, and *Plebeina*
71 *hildebrandti* are efficient pollinators of sweet melon, may be used in greenhouse crops.
72 Furthermore, several researchers have found the greater potential of stingless bee as pollinators
73 in crops under protected conditions.¹⁰ Honeybees are also not always the most efficient
74 pollinators due to a variety of variables, including body size and flower size mismatches, limited
75 nectar production, and specific pollen release mechanisms in some plants and when honeybees
76 fail to pollinate a crop efficiently, it's probably more cost-effective to look for a better pollinator-
77 plant match.

78 However, perusal of literature has shown scanty of information is available on pollination
79 of stingless bees on cucumber under protected condition in Assam. Thus, keeping in view the
80 importance of stingless bees in pollination, the present investigation had been worked out with
81 the aim of checking the efficiency of pollination of stingless bee, *T. iridipennis* in greenhouse
82 cucumber.

83 MATERIALS AND METHODS

84 **Study area:** The experiments were conducted during summer, 2020 and winter, 2020-2021 to
85 find out the pollination efficiency of *T. iridipennis* in cucumber under protected condition.
86 Summer and winter seasons were chosen because farmers grow their fruits in these two seasons
87 mostly in and around study area and it also helps them to get much more profit in both seasons as
88 it also grown as off season crop under green house condition. The study was conducted in the
89 Horticulture Experimental Farm, Department of Horticulture (N 26°43' and E 92°12') and
90 experimental field, All India Coordinated Research Project (AICRP) on Honeybees and

91 Pollinators, Department of Entomology (N 26°72' and E 94°19'), Assam Agricultural
92 University, Jorhat.

93 **Experimental design:** The experiments were conducted under two environments (open and
94 greenhouse) with three treatments: open pollination (OP), stingless bee pollination (SBP) and
95 pollinator exclusion (PE) with 30 replicates under each treatment (Here one plant denotes one
96 replicate). So total 90 plants were under observation. The SBP treatment was laid under
97 greenhouse condition in the Horticulture Experimental Farm. The OP and PE treatments were
98 laid in the experimental field under AICRP on Honeybees and Pollinators. For PE treatment, the
99 replicates were covered with customized nets of 2 mm mesh size to restrict the entry of
100 pollinators prior to flowering. A total of 70 m sq. area was covered under each treatment in both
101 seasons. No pesticides were applied in each treatment.

102 **Crop selection:** A local monoecious cultivar was selected for the cucumber cultivation under
103 each treatment and sown in both seasons for each treatment. The summer sowing of the crop was
104 done in April, 2020 and winter sowing was done in November, 2020 under both open and
105 protected conditions. The duration of the crop was 90-100 days. Various intercultural operations
106 viz., enriching the soil with vermicompost, irrigation and weeding were done on timely interval
107 during the crop growth period. The application of pesticides was strictly avoided.

108 **Installation of bee hive in green house:** One wooden box of 15504 cc (19×14×17 cc)
109 comprising approximately 700-1000 stingless bees, *T. iridipennis* was installed when 20-25%
110 plants were in flower (after 50 days of sowing) in the cucumber plants under SBP treatment in
111 both season and removed just before the harvesting period. ¹¹During the installation, one
112 adaptation method was followed proposed by Bartelli in 2014. One week after installation of

113 hive, when all plants bear flowers (almost 100%), bees acclimatized in the new environment and
114 started foraging on cucumber flowers and no bee mortality was observed.

115 **Foraging behaviour:** Observations on foraging behaviour were taken during the flowering
116 period of cucumber from morning to evening for 5 times (0600-0700, 0800-0900, 1000-1100,
117 1500-1600 and 1600-1700 hours) in a day. Here observations were not taken at midday time
118 interval (1100-1500) as because of high temperature, bees cannot forage. The foraging
119 parameters observed were: number of stingless bees visits per flower, time spent by stingless bee
120 (seconds) per flower and pollen load (mg). The number of stingless bee visits per flower was
121 observed at those time intervals for 1 minute by randomly selecting one flower from each
122 replicate for ten successive days in each season. So, total 300 minutes per time interval were
123 observed for ten days to observe the foraging behaviour. ¹²The time spent by a bee was recorded
124 on thirty flowers observed on each replicate in ten days with the help of a stopwatch followed by
125 the methodology of Raj and Rana in 2020. ¹³The observations on pollen load per trip were
126 recorded at those time intervals for ten days during the flowering period of cucumber following
127 the methodology of Erickson in 1973. At first ten incoming adult bees from the SBP plot were
128 caught by hand sweep net and then the pollen was removed from body as well as corbicula with
129 the help of a camel hair brush and collected on glass slide and weighed in an electronic weighing
130 balance. Ten adults were selected for measuring the pollen load because the weight of the pollen
131 of one stingless bee was negligible.

132 **Yield parameters:** Different yield parameters *viz.*, fruit set (%), average fruit length (cm), fruit
133 girth (cm), fruit weight (g) and yield per plant (kg) were observed from each replicate under
134 those three treatments and subsequently yield per hectare (t) was calculated after harvesting of

135 fruits in experimental plots for in both seasons. The percentage of fruit set was calculated by
136 following the formula:

$$137 \quad \text{Fruit setting (\%)} = \frac{\text{Number of fruits produced per plant}}{\text{Number of female flowers per plant}} \times 100$$

138 **Data analysis:** An analysis of variance (ANOVA) to compare the foraging activities between
139 summer and winter with a subsequent multiple comparison ¹⁴Tukey test (P<0.05) was performed
140 to compare the different yield attributes in different treatments (SBP, PE and OP).

141 **RESULTS & DISCUSSION**

142 **Foraging behaviour of *T. iridipennis* on cucumber**

143 Foraging activities of stingless bee (*T. iridipennis*) on cucumber were meticulously
144 checked during both seasons in SBP treatment. During the observation period, the results
145 revealed that the peak foraging time of *T. iridipennis* on cucumber flowers was 0800-0900 hours
146 in both the seasons. ^{15,16}Few scientists mentioned in their studies that peak foraging activity
147 period of *T. iridipennis* was recorded during 0800-1100 hours in Tamil Nadu and Karnataka of
148 India respectively. ^{17,18}Two similar studies on foraging activities of different stingless bees
149 (*Melipona quadrifasciata* and *T. miniangkabau*) on tomato and strawberry plant respectively
150 revealed that bees mostly visited the flowers of those plants during 0800-1100 hours under
151 greenhouse condition. The mean number of *T. iridipennis* workers per flower per minute was
152 found to be 0.73±0.21 during summer and 0.78±0.27 during winter. Also the highest number of
153 *T. iridipennis* was recorded during 0800-0900 hours in summer (1.06±0.15) and winter
154 (1.21±0.16). ¹⁹Few researchers also observed the maximum numbers of *T. iridipennis* per m sq.
155 per 10 minutes were 11 numbers during 0900-1000 hours in the morning and minimum of 2
156 numbers at 1700-1800 hours which also supported the present investigation. The average time

157 required by *T. iridipennis* for foraging per flower was recorded higher during winter season
158 (9.67 ± 1.61 sec) than summer season (8.71 ± 1.83 sec).¹⁹ Although the average time spent by *T.*
159 *iridipennis* was recorded higher in summer and winter comparing to the findings of who
160 recorded the lower average time spent by *T. iridipennis* (3.9 ± 0.5 sec in TNAU orchard and
161 3.5 ± 0.3 sec in Srivilliputhur). The probable reasons might be the differences in environmental
162 conditions. Observations on pollen load revealed that during summer, the average pollen load
163 carried by ten *T. iridipennis* bees was 4.63 ± 1.48 mg/trip and 4.80 ± 1.53 mg/trip during winter
164 (Table 1 and Figure 1).²⁰ The results slightly differed from the findings of who observed that the
165 average pollen load per stingless bee was 0.6 mg per trip *i.e.* 6 mg per 10 bees per trip in Africa.
166 The differences in the weather conditions (as in Africa, meteorological factors are completely
167 different from the present investigation site) of the experimental plots might have contributed to
168 these variations. The statistical comparison of three above mentioned foraging parameters
169 indicated a non significant difference between summer and winter season (Table 1). The foraging
170 patterns of *T. iridipennis* are depicted visually in Figure 2.

171 **Effect of *T. iridipennis* pollination on cucumber**

172 During summer and winter there were significant differences in fruit length, girth, weight
173 and fruit set of cucumber (Table 2).

174 During summer, the average fruit length of cucumber was observed significantly higher
175 in SBP plot (18.08 ± 1.23 cm) followed by OP (14.65 ± 0.94 cm) and PE (11.24 ± 0.79 cm) plots
176 (Table 2). Similarly, data on the highest fruit girth and fruit weight were recorded in SBP plot
177 followed by OP and PE (Table 2). The percentage of fruit setting was also observed significantly
178 higher in SBP ($79.06 \pm 8.60\%$) followed by OP ($66.03 \pm 12.69\%$) and lowest was recorded in PE
179 ($34.14 \pm 9.40\%$) (Table 2). A similar trend was also observed during winter, 2020-21 which is

180 presented in table 3 and 4. The fruit length, fruit girth and fruit weight were observed
181 comparatively higher in SBP plot followed by OP and PE plot. The highest percentage of fruit
182 setting was also observed higher in SBP plot ($83.85 \pm 6.16\%$) followed by OP ($67.75 \pm 8.01\%$) and
183 PE ($35.80 \pm 8.04\%$) plot (Table 2).

184 Significant difference in the yield per plant and yield per hectare was evident between all
185 the three treatments. The results pertaining to this experiment are presented in Table 3. In both
186 seasons, the highest yield was observed in SBP treatment followed by OP and PE treatment.
187 Accordingly, yield per hectare showed significant difference among all the treatments (Table 4).
188 A comparison of yield of these different treatments has been shown in Figure 3. After calculating
189 the yield increase of SBP over PE and OP during summer, it had been found that the yield
190 increase of SBP over PE was 512.37% meaning almost 5 times higher yield produced in SBP
191 plot comparing to PE and in case of yield increase of SBP over OP, almost 58.62% more yield of
192 cucumber produced in SBP plot compared to OP plot. During winter similar type of results
193 recorded for yield increases of SBP over PE and OP (Table 3). The results are in conformity with
194 the ¹⁶findings who observed significantly higher values of yield attributes in SBP plot when
195 compared to control plot in two different experimental locations (Coimbatore and Srivilliputhur
196 in southern India). ²⁰Some scientists have also been reported that the pollination by Brazilian
197 native stingless bee had contributed to a significant increase in fruit diameter and length of
198 cucumber as compared to the cucumbers pollinated by honeybees. The findings also suggested
199 that cucumber flowers pollinated by the pollinating agents such as bees have better qualities in
200 terms of weight, length and diameter. ²¹Furthermore, cucumber flowers pollinated by the
201 stingless bee may increase the quality of fruit production. ²²Few researchers, while investigating
202 the effectiveness of the stingless bees, *Scaptotrigona* aff. *depilis* and *Nannotrigona*

203 *testaceicornis* as pollinators of cucumber plants in greenhouses reported that the highest
204 cucumber yield was observed in those greenhouses that housed the stingless bees as pollinators
205 during the Brazilian winter season. ²The results of the experiments conducted by in Malaysia
206 also showed the cucumbers pollinated by stingless bees (*Heterotrigona itama*) produced heavier,
207 longer and larger cucumbers compared to those produced from pollination without stingless
208 bees. ²³Some reported that the pollination efficiency of *T. iridipennis* in watermelon where they
209 reported bigger fruit sizes and yield in case of bee pollinated crop comparing to control plot. ²⁴It
210 has also been reported the enormous yield increase of cherry tomato under protected condition
211 by using three native bees *i.e.* *Melipona bicolor*, *Nannotrigona testaceicornis*, and *Partamona*
212 *helleri* which proved as effective pollinators under protected condition. The bees visited the
213 flowers frequently and carried maximum pollen loads on corbicula present in their legs.
214 However, in PE plot where insect pollinators were restricted from visiting the flowers, limited
215 pollination occurred by the means of self and/or wind pollination, resulting in improper
216 pollination of flowers. This had also resulted in dropping off flowers, poor fruit setting and lower
217 yield. In OP plot, as the plants were grown under open environmental conditions, infestation of
218 insect pests was observed which resulted in lower yield compared to SBP plot. It has also been
219 cleared from (Fig. 4) that stingless bees acted as effective pollen collectors after observing the
220 transformation of pollen pots in hive which they collected from cucumber flowers during
221 flowering periods in pollinated cucumber plot.

222 It had been observed during the present investigation that *T. iridipennis* was readily
223 active as pollinator in cucumber flowers under protected condition during both summer and
224 winter season as because they swiftly adjusted to confinement, maintained high population
225 numbers, and displayed suitable foraging activities. As a result, *T. iridipennis* pollinated

226 cucumber plot produced 5 times more yield than pollinator exclusion plot. So one wooden hive
227 box (15504 cc) of *T. iridipennis* can be effective to pollinate cucumber flower of 70 m sq. area
228 under protected condition. We conclude that stingless bee (*T. iridipennis*) can be a very good
229 alternative as pollinator of cucumber flower under greenhouse condition where the use of other
230 honey bees or pollinators could be restricted and cucumber plant can be grown successfully as
231 off season fruit with the help of stingless bee in terms of pollination efficiency.

232

233 **AUTHOR CONTRIBUTIONS**

234 SS prepared the manuscript; SB checked the manuscript and PS helped to execute the
235 research work fruitfully. SS and SB performed the work and analyzed the data. Formatting was
236 done by RRT and PSS. Final checking was done by BB, SPN and MDR. All authors contributed
237 to the article and approved the submitted version.

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Table 1. Foraging behaviour of *Tetragonula iridipennis* on cucumber during summer (2020) and winter (2020-2021)

Time of observation (in hrs.)	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 ±0.21	0.78 ^a ±0.27	8.71 ^b ±1.83	9.67 ^b ±1.61	4.63 ^c ±1.48	4.80 ^c ±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

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

Treatment (N=30)	Avg. fruit length (cm)	Avg. fruit girth (cm)	Avg. fruit weight (g)	Avg. fruit set (%)	Avg. fruit length (cm)	Avg. fruit girth (cm)	Avg. fruit wt. (g)	Avg. 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 (t)	Yield increase over PE (%)	Yield increase over OP (%)	Yield/plant (kg)	Yield/ha. (Q)	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 the different letters shown in superscript are significantly different

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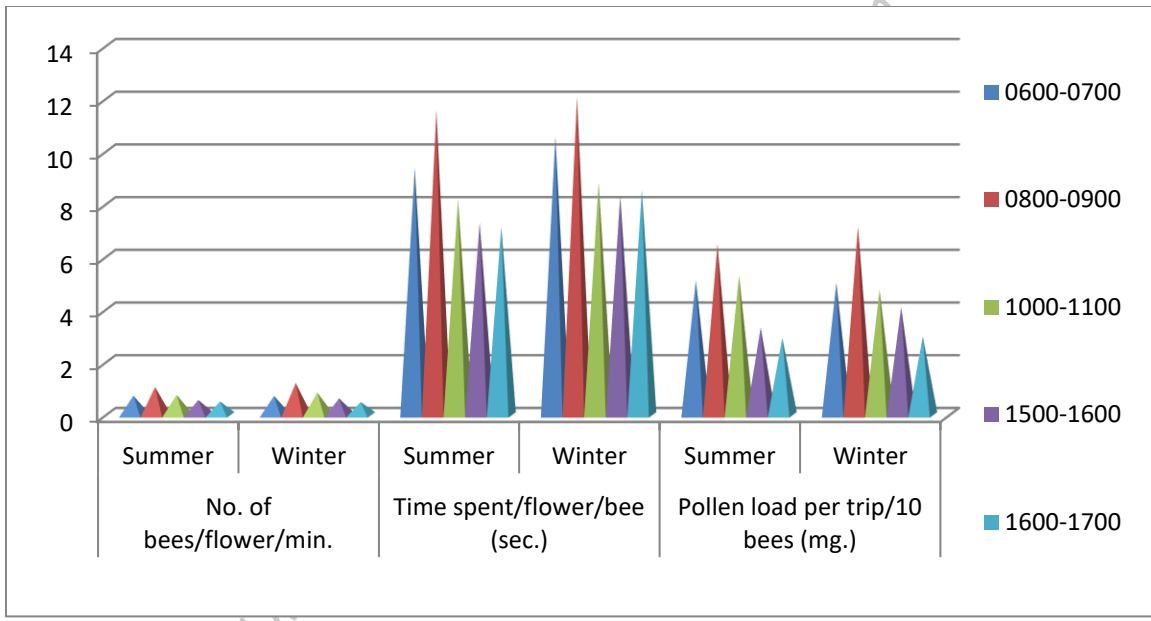


Figure 1. Foraging behaviour of *T. iridipennis* on cucumber during summer (2020) and winter (2020-2021)



A



B



D



C

Figure 2. Foraging pattern of *T. iridipennis* on cucumber flower; A: Initiation of foraging by 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

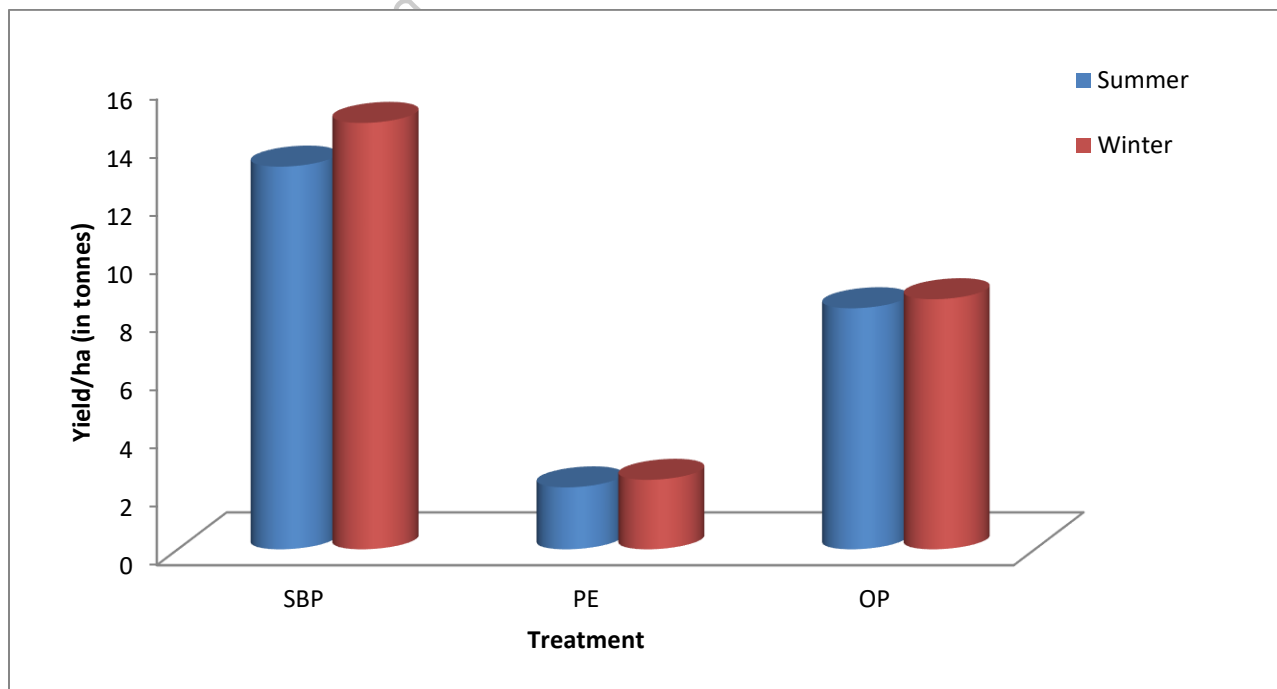


Figure 3. Yield of cucumber obtained under different treatments (t/ha) during summer (2020) and winter (2020-2021)

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on period



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Figure 4. Transformation of stingless bee hive after removing from SBP plot of cucumber after pollination period; A: Almost empty hive before installing into the greenhouse, **B:** Hive with filled up pollen, honey and brood area after stingless bee pollination

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