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POLLINATION POTENTIAL OF STINGLESS BEE *TETRAGONULA IRIDIPENNIS* SMITH IN ASH GOURD

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ABSTRACT

In Nagaland, farmers are practising stingless bee culture traditionally since time immemorial. Stingless bee *Tetragonula iridipennis* Smith is the most dominant of these along with *Lophotrigona canifrons*, *Lepidotrigona ventralis* and *Tetragonula laviceps* which are being reared in the Nagaland University, Medziphema campus. Ash gourd (wax gourd) (*Benincasa hispada)* is grown in Nagaland on a large scale and used as a vegetable. The present study is on the *Tetragonula iridipennis* and to ascertain its role as pollinators of ash gourd. The study was done under protected conditions, with four treatments- *T.iridipennis* pollination, *Apis cerana* pollination, open pollination and control (pollinator exclusion). Crop was raised as per good agricultural practices. Comparative foraging activity of the pollinators was observed. Colonies of *T. iridipennis* and *A.cerana* were introduced at 5% blooming stage in separate cages. The observations on relative abundance, foraging rate, foraging speed and loose pollen grains were made ascertain the pollination efficiency. Fruit production and quality parameters (fruit yield, fruit length, fruit diameter, fruit weight, Seed number, seed weight etc) were observed for each treatment. In control experiment, no pollinator was allowed to visit the crop. The evaluation of pollination potential revealed their effects on crop production and productivity. Data revealed many folds increase in fruit set (27.96%) and quality with *T. iridipennis* as compared to open and *A. cerana* pollination. Significantly the least fruit set (54.60%) was observed in without pollination. Thus, an increase of 634% was observed in increase in income of farmers.

Key words: *Tetragonula iridipennis*, *Apis cerana*, ash gourd, pollination index, protected conditions, pollination, yield and quality, cost benefit ratio

Many insects visit flowers to collect pollen and nectar as food, and pollination is an essential component of the ecosystem service (Costanza et al., 1997). Many flowers offer sugary liquid nectar as an added enticement for these pollinating insects. Among insect pollinators, bees are especially efficient and play a very important role in the pollination of flowering plants resulting in increased quantity and quality of fruits and seeds. They pollinate about 70% of crops that account for 35% of all agricultural production, and high-quality and high-quantity seed and fruit production (Klein et al., 2007 ; Kearns and Inouye, 1997). Collection and domestication of feral stingless bee colonies, their identification and use for pollination are the area of interest for sustainable farming in North East India. Propagation of stingless bee colonies contributes to preservation of biodiversity by conserving (Watanabe, 1994; Buchmann and Nabhan, 1996; Kearns and Inouye, 1997; Nabhan et al., 1998; Cane and Tepedino, 2001; Villanueva et al., 2005; Steffan-Dewenter et al, 2005 and Biesmeijer et al., 2006; Goulson et al., 2008).

The pollination potential of stingless bees in managed cropping system is still unexplored. Stingless bees are used as pollinator in greenhouse crops in both temperate and tropical regions (Sarto et al., 2005; Santos et al., 2009). Bumble bees, particularly *Bombus haemorrhoidalis* Smith, has been used in North India for greenhouse tomato (Yankit et al., 2018) and cucurbits pollination (Chauhan and Thakur, 2014; Kishan et al., 2017). However, in North East Region it is not widely used due to lack of colonies. Stingless bees being the native dominant pollinator can be better alternative as these are better pollinating agent due to its characteristics- viz., can pollinate small flowers, thrive much better in tropical areas, harm less to humans, coexist with other bee species, can adjust in confined space of greenhouse, net house conditions, more environment friendly, very less swarming ability, less chances of insect pests and disease attack and short foraging range etc. These can be used with cucurbits/ other crops in net house condition. (Gadhiya, 2015).

Ash gourd (*Benincasa hispida*) like other cucurbits require pollinators for fruit set (Roubik, 1995). Its seeds and seed oil are equally useful (https://www.lybrate.com/topic/ash-gourd). It is a highly cross-pollinated crop, and pollination plays a major role in increasing yield and quality. It is widely grown in Nagaland and whole North Eastern states as a delicacy and vegetable. Keeping in view these, effectiveness of different modes of pollination on ash gourd production and productivity to increase the farmer's income was evaluated in this study undertaken at Nagaland.

MATERIALS AND METHODS

The experiment was carried out at the Experimental farm AICRP Honey Bees and Pollinators, Department of Entomology, School of Agricultural Sciences and Rural Development (25.75961 °N, 93.853698 °E). Stingless bee *T. iridipennis* along with *Apis cerana* was explored for pollination in ash gourd under protected condition. Three naturally ventilated greenhouses (100 m^2 each) were selected and one *T. iridipennis* colony in greenhouse (T1) and similarly one *A. cerana* hive having 5 frames were introduced in second greenhouse (T2) at 5% flowering. The other greenhouse was kept as control (no pollinator was introduced, T3). Similarly, crop was grown under open condition for treatment T4. Whether these bees in greenhouse generated an effective pollination to improve fruit quality and quantity were studied.

All agronomical practices were done as per good agricultural practices, with the crop sown in the third week of July 2018. Total sixteen beds were raised in each greenhouse and seeds were sown at a spacing of 1 x 1 m. The crop came to bloom in the last week of September. After that the colonies were shifted in the greenhouse and data were recorded. Foraging activity of bees and other pollinators was recorded under open field conditions from early morning (800 hr) till late evening (1800 hr) at two hours interval for ten days continuously. The foraging activity was recorded as per the method adopted by Chauhan (2015). Pollination efficiency was derived for each species, using the formula suggested by Bohart and Nye (1960).

Impact of bee pollination on ash gourd was evaluated with % fruit set. The female flowers/ vine were precounted. Forty plants from each treatment viz., *T. iridipennis* pollinated, *A. cerana* pollinated, control and open pollinated were selected and tagged randomly. The fruit set on these plants were then recorded and total yield was calculated on fruit set basis, and % healthy fruits was also calculated. Similarly, % crooked fruits were calculated. A sample of 40 fruits were taken for calculating the fruit length, fruit diameter and fruit weight (10 fruits/ treatment i.e. *T. iridipennis* pollinated, *A. cerana* pollinated, control and open pollinated). The fruit length, fruit diameter and fruit weight were taken using the scale, digital Vernier callipers and digital weighing balance, respectively. Similarly, 40 mature fruits were taken (10 from each), seeds were removed and kept separately in water for 24 hr. After washing the seeds, these were dried in temperature controlled chambers for 24 hr, and then counted. Weight of 1000 seeds was also observed. The % increase in fruit set, healthy fruits, length, diameter, weight, number of seeds, weight of 1000 seeds was also calculated along with % decrease in crooked fruits. The data were statistically analysed with suitable transformation in R.B.D. design as per Gomez and Gomez (1986). Data on temperature and humidity was also recorded using the digital thermometer and hygrometer.

RESULTS AND DISCUSSION

Insect visitors under open conditions

Stingless bees (*Tetragonula iridipennis*, *Lophotrigona canifrons*, *Lepidotrigona ventralis* and *Tetragonula laviceps*), honey bees (*A. cerana, A. dorsata*, *A. florea*), xylocopa (*Xylocopa fenestrata* and *Xylocopa tenuiscapa*), halictid bees and megachilds were the most significant visitors visiting the crop throughout the day while syrphid flies and solitary bees were frequent visitors and blister beetle, grasshoppers and pumpkin beetles were recorded as less frequent on cucumber under open conditions (Table 1). Grewal and Sidhu (1978) also observed *A. florea, A. mellifera, A. dorsata, Halictus* sp. and *Bombus* sp. as major visitors of cucurbit crops. Similarly, Sajjanar et al. (2004) recorded 24 insects visiting cucumber crop in which hymenopterans were predominating.

Pollination efficiency

It was calculated on the basis of following attributes viz., relative abundance, foraging rate, foraging speed and loose pollen grains

Relative abundance: Data collected on pollinator activity presented in Table 2 reveal that stingless bees activity was maximum during 1000- 1200 hr, and *T.iridipennis* (11.10/ 5min/ m^2) significantly outnumbered the *A. cerana* (8.80 /5 min/m^2) and other pollinators (4.96 other pollinators/5 min/m^2).

Table: 1. Insect visitors of ash gourd under open conditions

S. No.	Scientific Name	Common Name	Order	Family	Frequency of occurrence
1.	*Tetragonula iridipennis*	Stingless bees	Hymenoptera	Apidae	M.F.V.*
2.	*Lophotrigona canifrons*				M.F.V.
3.	*Lepidotrigona ventralis*				MF.V.
4.	*Tetragonula laviceps*				F.V
5.	*Apis mellifera*	Italian honeybee	Hymenoptera	Apidae	M.F.V.
6.	*Apis cerana*	Indian honeybee	Hymenoptera	Apidae	M.F.V.
7.	*Apis dorsata*	Rock honeybee	Hymenoptera	Apidae	M.F.V.
8.	*Halictus sp.*	Solitary bee	Hymenoptera	Halictidae	F.V.
9.	*Episyrphus* sp.	Syrphid fly	Diptera	Syrphidae	F.V.
10.	*Musca* sp.	House fly	Diptera	Muscidae	LF.V.
11.	*Mylabris pustulata*	Blister beetle	Coleoptera	Meloidae	L.F.V
12.	*Monomorium indicum*	Ants	Hymenoptera	Formicidae	F.V.

MFV= Most frequent visitor; FV= Frequent visitor;LFV=less frequent visitor

Table 2. Relative abundance of different pollinators on ash gourd

S. No.	Time (h)	Number of foragers/ 5 min/ m^2			
		Stingless bee	Honey bee	Other pollinators	Mean
1	0800	4.16	2.22	1.66	2.68
2	1000	26.33	22.66	11.37	20.12
3	1200	14.33	12.33	6.44	11.03
4	1600	6.11	4.14	3.33	4.52
5	1800	4.58	2.66	2.00	3.08
Mean		11.10	8.80	4.96	

$CD_{0.05}$
Forager = 0.52
Time = 0.67
Forager x Time = 0.84

Maximum activity was recorded at 1000 hr (15.55 pollinators/ 5min/ m^2) and minimum (2.36 pollinators) at 1800 hr which was at par with that at 0800h (2.46 pollinators/5min/m^2). Roopa (2002) studied the seasonal variation in foraging activity of *T. iridipennis* Smith at Bangalore, and observed that during summer, major peak of pollen and nectar foragers were recorded between 1000 to 1200 hr and 1200 to 1300 hr and second peak between 1400 to 1500 hr and 1500 to 1600 h,r respectively. According to Devanesan et al. (2002), the foraging activity of *T. iridipennis* at Thiruvanthpuram started at 07 00 hr reaching its first peak at 12 00 hr. A decline was observed at 13 00 hr, and then reached second peak at 15 00 hr. There was almost no activity at 18 00 hr. Danaraddi (2007) observed the peak activity of *T. iridipennis* between 1000-1200 hr.

Foraging rate and speed/ loose pollen: Data gathered on foraging rate of different pollinators on ash gourd (Table 3) revealed that stingless bees significantly visited more flowers (7.59 flowers/ 5min) as compared to honey bees (5.64 flowers/ 5min) and other pollinators (4.59 flowers/ 5min). Devanesan et al. (2002), observed it at 07 00 h and maximum flowers for pollen were visited in the morning and early evening (Fidalgo and Kleinert, 2007). Table 4 reveals that foraging speed/ time spent was significantly less (4.15 sec/flower) compared to *A. cerana* (4.81 sec/flower) and other pollinators (8.11 sec/flower). Loose pollen grains were more with *A. cerana* (1970) on their body, compared to *T. iridipennis* bees (1564) and other pollinators (592) (Table 5). Table 6 indicates that *T. iridipennis* has maximum efficiency (24.00) as compared to honey bees (14.00), and is better in terms of Pollination Index.

Effect of mode of pollination

Length, diameter and weight of fruits: Significantly longer (38.00 cm) fruits were obtained from vines pollinated by T. iridipennis at par with the open pollinated ones (38.03 cm) which was followed by *A. cerana* pollinated (37.00) and minimum length

Table 3. Foraging rate of different pollinators on ash gourd

S. No.	Time (h)	Number of flowers visited by foragers/ 5 min by			
		Stingless bee	Honey bee	Other pollinators	Mean
1	800	6.33	5.33	2.66	4.77
2	1000	6.33	4.75	4.33	5.13
3	1200	7.33	5.02	2.97	5.10
4	1600	8.66	6.11	4.11	6.29
5	1800	9.33	7.00	8.88	8.40
	Mean	7.59	5.64	4.59	

$CD_{p=0.05}$

Forager	=	0.45
Time	=	0.45
Forager x Time	=	1.01

Table 4. Foraging speed of different pollinators on ash gourd

S. No.	Time (h)	Time spent (seconds) by foragers/ flower			
		Stingless bee	Honey bee	Other pollinators	Mean
1	800	5.1	6.66	12.60	8.12
2	1000	5.33	5.99	11.63	7.65
3	1200	5.1	4.75	3.66	4.50
4	1600	3.11	4.11	7.33	4.85
5	1800	2.11	2.55	5.33	3.33
Mean		4.15	4.81	8.11	

$CD_{0.05}$

Forager	=	0.59
Time	=	0.77
Forager x Time	=	1.33

Table 5. Number of loose pollen grains collected by different pollinators on ash gourd

S. No.	Number of loose pollen grains/ forager			
	Stingless bee	Honey bee	Other pollinators	Mean
1	1861	2130	661	1550.66
2	1178	1910	530	1206
3	1653	1870	585	1369.33
Mean	1564	1970	592	

Table 6. Comparative pollination efficiency of different insect pollinators on ash gourd

S. No.	Pollinator	Pollination index/ efficiency parameters				
		Relative abundance	Foraging Rate	Foraging Speed	Loose pollen grains	Pollination Index
1	Honey bee	8.80 (2)*	5.64 (2)	4.81 (2)	1970.00 (3)	14
2	Stingless bee	11.10 (3)	7.60 (3)	4.15 (3)	1564.00 (2)	24
3	Other pollinators	4.96 (1)	4.59 (1)	8.11 (1)	592.00 (1)	3

*Value in parenthesis are ranks assigned of 17.96 cm in pollinators excluded block (control). Similarly, significantly increased weight (3.366 kg) and diameter (13.13 cm) of fruits was observed with *T. iridipennis* at par with the fruit weight (3.248 kg) in open pollination conditions. (Table 7; Fig. 1).

Seed number/ weight: Bee pollinated flowers yielded fruits having significantly more seeds viz., 1295 and 1346/ fruit, while it was 988 in open pollinated plots. Similarly, the weight of 1000 seeds was significantly more (41.24 g) followed by 39.60 g, 34.00 g and 16.90 g extracted from fruits of *T. iridipennis*, *A. cerana* and, open pollinated and control plots (Table 8; Fig 2).

Fruit yield and quality: Higher fruit set (82.56%) was recorded with *T. iridipennis* significantly more compared to that of *A. cerana* (79.66%) and open pollinated crop (61.46%). Similarly significantly least crooked fruits (13.73%) were obtained when T. iridipennis was used. Significantly more healthy fruits (86.27%) were produced from *T. iridipennis* pollinated plots (Table 9).

Productivity and quality: Use of *T. iridipennis* led to increase in the fruit set and healthy fruits were found to be 27.96 and 28.93% over the control, and a reduction in crooked fruits (141.01%) was observed (Table 10). The cost benefit ratio was found to be 1:1.2 in ash gourd when grown under protected conditions and use bees as pollinators (Table 11).

A research trial on watermelon in Florida showed that the number of bee visits was more important than the length of time that each bee stayed on the flower. Well-shaped, fully-expanded fruit developed after eight bee visits to a female flower. Fruit set was significantly reduced when bees only visited two to four times. Misshaped or undersized fruit could be the result of poor or incomplete pollination (Westerfield, 2014). Occhiuzzi (2000) reported that *Trigona carbonaria* Smith effectively pollinated sweet pepper under glass green house conditions in Australia. Fruit weight had increased by 11% and number of seeds/ fruit by 34% compared to crops that were not pollinated by bees. Similarly, Santos (2004) reported that *Scaptotrigona* aff. *depilis* and *N. testaceicornis* Lepeletier effectively pollinated greenhouse cucumber in Brazil, resulting in a higher fruit production, higher fruit weight and more of perfect fruits compared to the control, where no pollinators were present.

Sarto et al. (2005) reported that greenhouse tomatoes pollinated by *M. quadrifasciata* Lepeletier had greater fruit set and better fruit quality. However, in that study, the control flowers were bagged, resulting in complete failure of fruit set. Santos et al. (2009) reported that the stingless bees pollination in green house yielded 1414 tomato fruits as compared 1220 fruits in green house with honey bees, 1187 fruits in green house with control and 730 fruits in open area. Titayavan (2010) studied the pollination efficiency of two different *Trigona* species at Thailand. Rajasri et al. (2012) studied effect of bee pollination on seed yield of sunflower and reported that seed yield of sunflower/ 30 plants was 633 g in treatment of pollination by *T. iridipennis* while it was only 352 g

Table: 7. Effect of different modes of pollination on fruit length, diameter and weight in ash gourd grown under protected conditions

Pollination Type	Length (cm)	Diameter (cm)	Weight (g)
Stingless bee*	38.00	13.13	3.366
Honey bee**	37.00	12.40	3.087
Open	38.03	12.76	3.248
Control	17.96	7.40	0.381

*Stingless bee = *Tetragonula iridipennis*; **Honey bee= *Apis cerana*

Table: 8. Effect of different modes of pollination on seed number and seed weight in ash gourd

Mode	Seeds/fruit (Number)	Weight (1000 seeds in grams)
Stingless bee*	1295 ± 66	41.24
Honey bee**	1346 ± 52	39.60
Open	988 ± 61	34.00
Control	212 ± 17	16.90

*Stingless bee = *Tetragonula iridipennis*; **Honey bee= *Apis cerana*

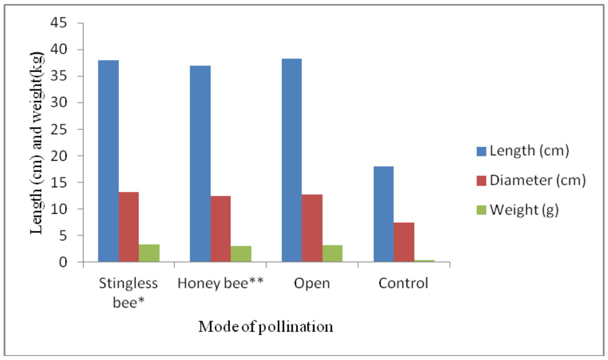

Fig.1. Impact of different modes of pollination on ash gourd quality parameters

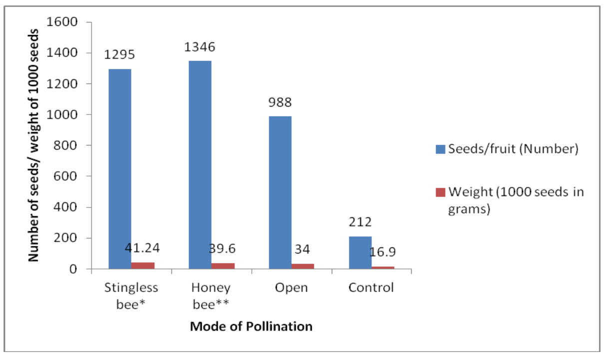

Fig.2 Impact of stingless bee pollination on ash gourd seed numbers and seed weight over control

Table: 9. Effect of different modes of pollination on fruit set and quality in ash gourd

Pollination	Fruit set (%)	Crooked fruits (%)	Healthy fruits (%)
Stingless bee*	82.56	13.73	86.27
Honey bee**	75.12	20.34	79.66
Open	62.90	38.54	61.46
Control	54.60	33.09	66.91

*Stingless bee = *Tetragonula iridipennis*; **Honey bee= *Apis cerana*

Table .10. Per cent increase in the quality and production of ash gourd grown under net house conditions through stingless bee pollination over control

Quality Parameters	Per cent increase
Fruit set	27.96%
Healthy fruits	28.93%
Crooked fruits*	141.01%
Fruit length	111.58%
Fruit diameter	77.43%
Fruit weight	783.46%
Seed number	1083 %
Weight (1000 seeds)	144.02%

*Reduction in percent of crooked fruits

Table 11. Cost benefit ratio of ash gourd

Yield per plant = 10.50 kg
Yield/plot = 63 kg
Total yield per net house = 1008 kg

Variable cost (Rs.)		Fixed Costs (Rs.)	
Human labour	3000	Management cost	1000
Land preparation	500	Risk margin	500
Nursery Raising	100	Total fixed cost	1500
Transplanting	100		
Weeding	1000		
Irrigation	400		
Inter-culture operations	1000		
Harvesting	500		
Seed	150		
Fertilizer	3000		
Crop protection	400		
Miscellaneous	2000		
Total variable cost	12150		
Total input cost= fixed cost + variable cost			13,650/-

Total Output	1008 kg
Total returns @ Rs. 30/kg	30,240
Returns over total cost = Total returns-Total cost	16590
Input : output ratio = Output/input	1:1.56

Cost benefit ratio : 1: 2.2

Stingless bee pollination

Yield/plant = 16.80kg
Yield/plot =100.80kg
Total yield from stingless bee pollinated plots = 1612.80kg
Total returns @ 30Rs/kg = 48,384

Control

Yield/plant = 2.286kg
Yield/plot = 13.72 kg
Total yield from control plots = 219.52kg
Total returns@ 30Rs/kg = 6585.60Rs

Difference between Stingless bee pollinated and control returns = 41798.40 Rs.

A difference of 634 % increase was observed in the total returns in stingless bee pollinated ash gourd than control.

in self pollinated crops. Viana et al. (2014) found that stingless bee *Mandacaia* (*Melipona quadrifasciata anthidioides* Lepeletier) play an important role as pollinator of apple flowers.

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