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First record on quantification of nectar and pollen content in the larval provision of stingless bee, *Tetragonula iridipennis*, Smith

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Abstract
Tetragonula iridipennis Smith, also known as dammer bee is happens to be the only species of stingless bees found in most of the North Indian states. In the present study, a quantitative assessment of the larval provision was done for realizing the total pollen and nectar content consumed by a *T. iridipennis* worker bee, during its development. The eggs were removed by decapping the *T. iridipennis* brood cells to suck the total larval provision from 60 brood cells. After collecting the larval provision in 6 Eppendorf tubes, each containing larval provision from 10 brood cells, the total dry pollen content was weighed after the complete dehydration. The average dry pollen content present in all the 60 brood cells was calculated to be 7 mg as compared to the 70 mg of total moisture content. Further, the estimation of total moisture content present in recently collected pollen by these bees, gave the fraction of pollen (10.51%) and nectar (89.48%) present inside a single brood cell of *T. iridipennis* bees.

Keywords: Stingless bee; *Tetragonula iridipennis*; quantitative assessment; larval provision; brood development

1. Introduction
Proper nourishment during the early developmental phases plays a key factor in the ultimate survival of newly emerged young ones in most animals. Unsurprisingly, the importance of this nourishment becomes more evident in those eusocial insect species, where it regulates the factor of caste determination in the family.

Most if not all the pollinator species depends solely upon the nutrients coming from the nectar and pollen of the plants which they pollinate upon. Normally, while most of the pollinator bee species gets a large chunk of their dietary carbohydrate requirements from nectar, the other portion of the nutritional requirements are provided through the pollen [1-3]. Many bees mix this pollen and nectar to feed their immature stages, kept inside the different shapes and sizes of rearing chambers or compartments. The method of providing this mixture or provision to their immature stages varies in different bee species. Unlike the well known progressive feeding method utilized by *Apis* bees, the stingless bees employ the mass provisioning method to feed their developing larvae [4-7]. Like most of the solitary bee species, stingless bees (Meliponini: Hymenoptera), fills the rearing chamber or brood cells with the larval provision and supply each brood cells with an egg. After emergence the developing larvae feed onto this already supplied larval provision and thus do not require any secondary input after the deposition of an egg inside the brood cells [8].

At once this mass provisioning of larval provision seems to be advantageous to the stingless bees, as the total development takes place in a concealed environment without the interferance of any adult bee. However, due to the development taking place under a sealed environment, it becomes impossible for an adult stingless bee to assess any response from developing larvae, regarding a nutritional imbalance or deficiency [9].

Being considered as generalist pollinators, stingless bees are supposed to be in constant contact with different pesticidal compounds [10]. With an ever increasing level of agrochemical inputs, the problem of pesticidal exposure becomes more hazardous on different colony levels including brood developing in the colony [11].

The nutritional quality of larval provisions gets affected by a wide array of sources, starting from the lower quantity of pollen to the inclusion of contaminated pollen in the provision. Thus, considering the nutritional and toxicological importance of the constituents of the larval

provision in stingless bee larval diet, quantification of the total larval provision is of great importance.

Only a total of eight species of stingless bees are found in the Indian sub-continent, with more diversity being, distributed around the south Indian states like Andhra Pradesh, Karnataka, Kerala and Tamil Nadu [12]. Some other states in North-eastern India such as, Nagaland, Arunachal Pradesh and Assam also have been reported to be housing as many as three different species of these bees. Out of all eight species, reported from India, *Tetragonula iridipennis*, Smith or commonly known as dammer bee or Asiatic stingless bees are the most widely distributed stingless bees in the country. *Tetragonula iridipennis* is the only stingless bee species distributed in the comparatively colder and drier states of North India and is also the only stingless bee species present in Uttarakhand, one of the North Indian states at a higher elevation.

Being domesticated for centuries in different parts of the globe, information regarding the quantitative or qualitative characteristics of the larval provision in stingless bee species is still lacking. Further, it will be the first record on the quantitative analysis of larval provision of stingless bee, *T. iridipennis*. The results of this study will provide an insight into the fraction of nectar and protein rich pollen content present in the larval provision of stingless bees will give an advantage for different toxicological bioassays and *in vitro* production of these bees. Thus, in order to assess the prospects of preparing a synthetic diet for different bioassays and *in vitro* production strategies, we have focused on to the quantificational investigation of *T. iridipennis* total larval provision per brood cell.

2. Material and Methods

The present investigation was done to quantify the pollen and nectar content present inside a worker bee brood cell of stingless bee *T. iridipennis*. The study was done at the Department of Entomology, G. B. Pant University of Agriculture and Technology, Pantnagar. During the present study, a quantification trial was conducted for determining the amount of pollen and nectar percentage in larval provision, provided in the natural conditions by these bees. This would suggest the amount of pollen and nectar ingested during the developmental period by a *T. iridipennis* larva.

2.1 Collection of natural *Tetragonula iridipennis* brood cells

For the present study, a strong stingless bee, *T. iridipennis* colony was selected in the honey bee research and training centre of the university. Further, about 80-100 newly sealed natural worker brood cells were collected from this colony with the help of a sharp knife and these brood cells were brought to the laboratory inside a collection box, having moist cotton and muslin cloth at its base.

2.2 Collection of larval provision

In the laboratory, the larval provision was sucked from 60 different *T. iridipennis* brood cells, by using microcapillary tubes (10 µL). This was done by opening the operculum of sealed brood cells by gently scrapping the surface with the help of a fine needle, while avoiding any damage to the egg. Further, these eggs were removed from the brood cells under a stereo zoom microscope with the help of a fine needle, prepared by joining a micro pin to the handle of a paint brush. Larval provision was then sucked with the help of a microcapillary tube (20 µL), jointed to a catheter pipe for easy suction of the viscous larval provision. This provision was collected in an eppendorf tube and homogenized on a vortex mixture for further use. Larval provision from 10 brood cells was collected in an eppendorf tube, containing 1 ml of water. Thus larval provision from 60 brood cells was distributed in 6 Eppendorf tubes, with each having provision from 10 brood cells.

2.3 Determination of nectar and pollen fraction present in the larval provision

The weight of each microcapillary and eppendorf tube, before and after the sucking of larval provision from 60 brood cells was recorded using an analytical weighing balance. This gave the total weight of larval provision, inside a single brood cell.

Further, this mixture of larval provision and distilled water from each of the 6 eppendorf tubes was filtered with the help of a Grade-1, qualitative filter paper (HIMEDIA Laboratories, pore size: 2.5 µm) and subsequently oven dried at 60ºC, until complete dehydration. The weight of filter paper before and after the dehydration was recorded to determine the amount of dry pollen content in a single brood cell. In addition to this, moisture content of a natural pollen sample was estimated to get the actual amount of nectar present in the larval provision. Pollen moisture, present in the natural pollen sample, collected from the *T. iridipennis* colonies was calculated by completely dehydrating the known weight (2 gm) of natural pollen at 60ºC for one hour. The weight loss by the pollen after oven drying was considered to be the amount of pollen moisture, present in the natural pollen. Quantification of the exact amount of moisture and dry pollen content in a natural pollen sample, gave the fraction of natural pollen and nectar content mixed in the larval provision per brood cell.

3. Results and Discussion

After deducting the weight of microcapillary filled with larval provision from the weight of empty microcapillary for each of the 60 natural brood cells, the average weight of larval provision in 60 natural brood cells of *T. iridipennis* bees was found to be 7.7 mg (Table 1). The larval provision, extracted from 10 brood cells, kept in a single eppendorf tube containing 1 ml water in it was purified with the help of a filter paper.

Table 1: Weight of larval provision sucked from 10 brood cells.

S. No	Wt. of total larval provision in mg [wt of empty microcapillary (E)- wt of provision (LP)] (LP10)					
	R_1 (E=369)	R_2 (E=341)	R_3 (E=359)	R_4(E=342)	R_5(E=339)	R_6(E=365)
1	(378-E) = 9	(350- E) = 9	(366- E) = 7	(348- E) = 6	(348- E) = 9	(370- E) = 5
2	(379- E)= 10	(349- E) = 8	(367- E) = 8	(351- E) = 9	(348- E) = 9	(372- E) = 7
3	(378- E) = 9	(349- E) = 8	(369- E) = 10	(348- E) = 6	(346- E) = 7	(374- E) = 9
4	(375- E) = 6	(349- E) = 8	(367- E) = 8	(349- E) = 7	(346- E) = 7	(376- E) = 11
5	(375- E) = 6	(350- E) = 9	(367- E) = 8	(350- E) = 8	(347- E) = 8	(371- E) = 6
6	(378- E) = 9	(351- E)= 10	(367- E) = 8	(350- E) = 8	(347- E) = 8	(370- E) = 5
7	(375- E) = 6	(347- E) = 6	(366- E) = 7	(351- E) = 9	(345- E) = 6	(373- E) = 8

8	(376- E) = 7	(349- E) = 8	(366- E) = 7	(350- E) = 8	(347- E) = 8	(375- E) = 10
9	(378- E) = 9	(347- E) = 6	(367- E) = 8	(349- E) = 7	(346- E) = 7	(372- E) = 7
10	(376- E) = 7	(349- E) = 8	(366- E) = 7	(350- E) = 8	(346- E) = 7	(371- E) = 6
Total	78	80	78	76	76	74

After drying in the hot air oven, the average weight of dry pollen in the six sets, in which each set containing larval provision from 10 natural *T. iridipennis* brood cells was found to be 7 mg (Table 2). Thus, declaring the weight of dry pollen in each brood cell as 0.7 mg.

Table 2: Weight of dry pollen and total liquid phase (in mg.), present in the larval provision of a worker bee brood cell of *T. iridipennis*

Replication	Wt. Of empty filter paper (FP)	Wt. of dry pollen + FP (PFP)	Wt. of dry pollen (PFP-FP)	Total liquid phase (LP10-wt. of dry pollen)
R_1	472	480	8	70
R_2	474	480	6	74
R_3	483	493	10	68
R_4	408	418	10	66
R_5	579	584	5	71
R_6	575	578	3	71
Mean			7	70
STD±			2.82	2.75

As the natural pollen contains a variable amount of pollen moisture; thus, in order to determine the moisture content in the natural pollen stored in a *T. iridipennis* colony, the pollen sample was weighed before and after the oven drying in a filter paper. The average weight of 6 pollen samples (total weight= 2 gm each), collected from the natural colonies after the oven drying was recorded to be 1686.50 mg (Table 3); whereas, the average total moisture content in these samples was found to be 313.50 mg. Therefore, depicting the average percent moisture content of a natural pollen sample (2.00 gm), as 15.72%. Consequently, after adding up the amount of moisture content in each of the average weight of dry pollen per 10 natural *T. iridipennis* brood cells, the average weight of pollen content per 10 brood cells was found to be 8.10 mg; which made the weight of pollen content as 0.81 mg per brood cells; while the amount of nectar content in each brood cell was calculated as 6.89 mg. Thus, the percentage of natural pollen and nectar content in the 7.7 mg of larval provision in a single brood cell was determined as 10.51 and 89.48%, respectively.

Table 3: Weight of pollen moisture (in mg.), present in the recently collected pollen samples in the stingless bee, *T. iridipennis* colony.

Replication	Wt of Empty filter paper	Wt. of Filter paper + Pollen sample (EFP+P)	Wt. of dried EFP+P	Wt. of dried pollen in sample	Wt. of pollen moisture content	Percent pollen content (%)	Percent nectar content (%)
R_1	627	2627	2313	1686	314	84.30	15.70
R_2	628	2628	2315	1687	313	84.35	15.76
R_3	629	2629	2316	1687	313	84.35	15.76
R_4	626	2626	2312	1686	314	84.30	15.70
R_5	627	2627	2313	1686	314	84.30	15.70
R_6	626	2626	2313	1687	313	84.35	15.70
Mean	627.17	2627.17	2313.67	1686.50	313.50	84.33	15.72
STD±	1.17	1.17	1.51	0.55	0.55	0.03	0.03

Variable amount of larval provision and accordingly variable amount of pollen content, inside a single brood cell for four stingless bee species have been reported by Rosa *et al.* [13]. The amount of larval provision and pollen content in the same was found to be 9.4± 0.0001 and 1.3 ± 0.000; 49.8± 0.0010 and 6.0± 0.0003; 37.3± 0.0006 and 1.9± 0.0001 and 10.1± 0.0002 and 0.4± 0.000 mg for four stingless bee species, namely *Plebeia droryana*, *Melipona obscurior*, *Scaptotrigona bipunctata* and *Tetragonisca fiebrigi*, respectively. The caste differentiation in stingless bees is known to be depending upon the trophic difference among the developing larvae, [4-7, 14]. Thus, the quantitative analysis of their larval provision can be helpful in the *in-vitro* production of these bees. Further, the studies on the chemical composition of this larval food will serve as a milestone for the preparation of an artificial diet for rearing of these bees for the better management and different toxicological bio-assays for assessment of pesticidal toxicity to different beneficial insects.

4. Conclusion

Rearing of developing young ones with the mass provisioning method, inside a closed brood chamber in different stingless bee species provides a great opportunity of rearing these bees in controlled conditions. Thus, the outcome of this investigation on the quantitative analysis of pollen and nectar content in a *T iridipennis* brood cell should provide a foundation for further studies on development of artificial diets for the controlled rearing of these bees. Further, the dependency on nutritional difference for the caste differentiation in these bees also advocates for the further exploitation of such artificial diets in the *in vitro* rearing of queen bees for realizing the commercial potential of these bees.

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