Composition and nutrition of wild bees in *Solanum melongena* L. agroecosystems in Pune, Maharashtra, India.

ABSTRACT

Eggplant, (*Solanum melongena* L.), also called aubergine or Guinea squash, in the nightshade family (Solanaceae) is an economically important crop in India. We investigated the significance of *Solanum melongena* pollen in the diet of wild bees found in agroecosystems by examining pollen on the bees using a pollen load analysis and a nutritive analysis. We selected five agricultural sites that cultivate *S. melongena* near Pune, Maharashtra, India. At each site, over a two-year period, we sampled the wild bees that visited mature flowers. We sampled pollen from mature flowers and also from the bodies of the bee specimens. The pollen grains from the bees were observed under a light microscope and a scanning electron microscope. They were counted using a Neubauer chamber and the pollen probability index for each bee species was calculated. The nutritive value of *S. melongena* pollen was estimated by extracting and characterising their proteins using liquid chromatography mass-spectrometry. Protein peptides sequences were extracted from the NCBI protein database to examine their essential amino acids. We collected 324 bees representing 11 species in three families involved in *S. melongena* pollination. *Apis florea* and *A. cerana* were the most abundant bees that visited *S. melongena* flowers. Pollen load size is highly variable ranging between few thousand to 134146 pollen grains per bee. However, the pollen probability index indicated a high degree of specificity to *S. melongena* pollen. *S. melongena* has high pollen protein content and a total of 10 different proteins were identified that are important for plant cell activities as well as the nourishment of pollinators. Further proteomic characterization of the indicated that nearly half the essential amino acids played and nutrient role in the bees. This study highlights that nutrititive composition of *S. melongena* pollen and highlights its potential to play a significant role in wild bee diets.

Keywords: Pollen, *Solanum melongena*, Wild bees, Pollen nutritive value; LC-MS/MS

1. INTRODUCTION

Wild bees are critical for the pollination of fruit and vegetable crop species in agroecosystems [1]. Bees deliberately seek out and collect pollen in addition to nectar for nutrition. Bees exhibit flower constancy, i.e., on a given foraging trip, individual bees focus exclusively on a single plant species making both foraging and pollination efficient [2]. Bee visitation rates to flowers depend upon olfactory and visual cues such as floral scents, color, symmetry, shape, and ultraviolet landing strips [3,4]. Because of their nutritional needs, bee foraging visits can also be influenced by the chemistry of the pollen. There is some evidence that honeybees, bumblebees, and wild bees will seek out plant species with greater protein content in their pollen, possibly even adjusting their foraging behaviour to suit this preference [5,6].

Pollen grains consist of proteins, lipids, carbohydrates, vitamins, flavonoids, and carotenoids [7]. Pollen is the sole source of protien for bees and pollen protein content can vary from 12% to as much as 60% among various plant species. Literature reviewed by [8] indicated that pollen protein is critical to adult survival, brood size and rearing, immunocompetence, and ovary and hypopharyngeal gland development in honeybees. Similar studies in bumblebees have demonstrated the importance of these proteins for colony development [9, 10]. However, very little has been explored about wild bee nutrition [11], especially in the context of crop species in agroecosystems.

Pollinator preference for protein rich pollen can have broader evolutionary implications [12]. For example, members of the genus *Solanum* (family Solanaceae) have evolved poricidal anthers as part of a pollination syndrome involving bees. Bees sonicate the poricidal anthers by buzzing, milling, and even biting, inducing pollen release [13]. Populations of the wild *Solanum lycocarpum* in Brazil help maintain wild bee local communities [14].

The genus *Solanum* has yielded important crop species including tomatoes (*Solanum lycopersicum* L.), potatoes (*Solanum tuberosa* L.), and eggplants (*Solanum melongena* L.). These crop species could play an important part in the diet of local wild bees in the areas that they are cultivated. *Solanum melongena* is an important crop in both temperate and tropical areas; about 50 million tonnes of the fruit are produced globally every year. In India, it is the third most important vegetable crop after tomatoes and potatoes. In 2013 alone, 310 million hectares of agricultural land in India was devoted to cultivating *S. melongena*, yielding 8.3% of the total vegetable crop production nationally [15].

Given the large area devoted to *S. melongena* cultivation in India*,* we hypothesized that it is likely a significant source of nutrition for native wild bee communities in agroecosystems. *Solanum melongena* flowers are blue, bisexual and odorous with poricidal anthers. Studies have indicated that pollination by bees is important for fruiting and seed set in the cultivated species and its wild relatives [16].

In this study, we investigated the significance of *S. melongena* pollen in the diet of wild bees found in agricultural areas near Pune, Maharashtra, by examining pollen on the bees using a pollen load analysis and a nutritive analysis. Our study highlights the nutritive role that a crop species, such as *S. melongena,* significant to human consumption can play in wild bee diets

2. MATERIAL AND METHODS

2.1 Study Area

Our study was conducted in the outskirts of Pune, Maharashtra, a fast-growing urban centre in India located at the foothills of the Western Ghats, a region recognized as a tropical biodiversity hotspot. The area alternates between four months of monsoon and eight months of a dry period. Pune city is surrounded by an agricultural landscape where sugarcane (*Saccharum officinale*), a major cash crop, is grown along with several types of cereals, grains, and vegetables. *Solanum melongena* (eggplant) is cultivated all year long, both as a summer and a winter crop in the region.

We selected five agricultural sites that cultivate *S. melongena* in the agricultural areas. The sites were located at a distance of at least 3 km from each other. The *S. melongena* crop fields sampled for the study were surrounded by wheat (*Triticum indicum*), ground nut (*Arachis hypogaea*), jowar (*Sorghum bicolor*), and onion *(Allium cepa)* fields.

2.2 Sampling Bee Species and Pollen

Each site was visited for a total of ten days over a two-year period. Sampling was carried out on sunny days between 10:30 am and 1:00 pm when bees are most active. At each site, we walked a transect of 50 m along a crop row and randomly selected a flower in complete anthesis on five individual *S. melongena* plants. Each flower was observed for 15 minutes and hand nets were used to capture bees visiting the flowers. Bee specimens were placed in individual vials after being euthanized in killing jars containing ethyl acetate and were taken to the lab for identification and retrieval of pollen on their bodies. Mature pollen was also collected directly from mature of *S. melongena* flowers for reference.

2.3 Bee Identification and Pollen Retrieval

At the lab, the bee specimens were examined under a dissecting microscope and identified using the dichotomous keys in Michener (2007). Specimens are deposited at the Department of Zoology at Savitribai Phule Pune University, Maharashtra, India. Pollen contained on the bee specimens were removed and stored at - 20 °C for further analyses. Mature pollen collected directly from *S. melongena* flowers for reference was also stored at - 20 °C.

2.4 Pollen Quantification

We used both light microscopy and field emission scanning electron microscopy (FESEM, FEI Nova SEM™ 450) to examine the topology of the pollen. Pollen grains were washed with absolute ethanol and acetolysed before being examined at 40 x and 100 x magnifications (Zeiss A X10). For FESEM, acetolysed pollen were treated with HMDS (hexamethyldisilazane), mounted on carbon tape, sputter-coated with platinum, and imaged at 15 kv.

The morphology of a pollen grain is unique to its corresponding plant species. Therefore, pollen retrieved from the bodies of bees can be used to identify plant species that serve as their food source [18]. Pollen grains of *S. melongena* on bee specimens were identified by comparing their morphology with those gathered directly from mature flowers of the plant. To quantify pollen grains collected from each bee specimen, we first diluted 10 mg of a pollen pellet in 1 ml distilled water and then counted the grains under a light microscope at 40 x magnification using a Neubauer chamber.

2.5 Pollen Load Analyses

We conducted the pollen load analyses using the pollination probability index (PPI) developed by [19] which incorporates the proportion of pure to mixed pollen loads, or the average percentage of conspecific pollen on bees and reflects floral constancy at the pollinator level. The pollination probability index (PPI) is calculated as: *PPI = PCP x PBP* where PCP is the mean proportion of conspecific pollen of *S. melongena* in the total pollen load of each bee, and PBP is the proportion of bees out of the total number of bees sampled carrying that conspecific pollen. We used a Kruskal-Wallis nonparametric test to determine statistically significant variation in the pollen load among the different bee species. Statistical analyses were conducted using PAST software Version 3.20 [20].

2.6 Pollen Protein Analyses and Nutritive Value of Proteins

We followed [21] for pollen protein extraction. Total proteins were quantified using methods outlined by [22] with BSA (bovine serum albumin) as a standard. Proteins were characterized using liquid chromatography mass-spectrometry and MALDI TOF (matrix-assisted laser desorption/ ionization-time of flight) techniques.

Samples were prepared for MALDI TOF by mixing 20 µl of the crude protein extract with acetonitrile in a 1:1 ratio. The aliquot was incubated for 20 minutes at room temperature after which 40 µl of DTT (dithiothreitol; Cleland's reagent) was added to it and the resulting mixture was heated to 60º C for 10 minutes before cooling for 15 minutes. 20 µl of iodoacetamide was added to the cooled sample and the resulting solution was incubated for 35 minutes at room temperature. 20 µl of freshly prepared trypsin (0.3% trypsin:protein ratio, w/w) was added to the solution and further incubated at room temperature for 10 minutes. The suspension was diluted with 25 mM ammonium bicarbonate to a concentration of 5 mg/µl and incubated for 4 hours at 37º C. After incubation, 20 µl of 3% formic acid was added to the solution. This process allowed for completion of protein digestion.

20 µl of the digested protein was injected into the LC-MS column. To identify peptides, Proteome Discoverer™ (Thermofisher Scientific), operated on a local server, was used to search the NCBI database (Figure 1). The high-scoring peptides corresponded with the peptides that were above the threshold in our Proteome Discoverer search (expected $p < 0.05$). Protein peptides sequences were extracted from the NCBI protein database to examine their essential amino acids. The nutritive value of each protein was estimated and calculated by using the algorithm by [23]: *Nutritive Value of a Protein = (Number of Essential Amino Acids / Number of Total Amino Acids) *100.*

3. Results

3.1 Bee Species Visiting *S. melongena* Flowers

We collected 324 bees representing 11 species in three families (*viz.* Apidae including tribe Anthophorini, Megachilidae, and Halictidae) that are involved in *S. melongena* pollination. Seven species from the family Apidae represented about 50 % of the individuals collected. The tribe Anthophorini made up 4% of the bees collected but was represented by only a single genus.

Apis florae was the most abundant species (33%) along with *A. cerana* (10%). Other species that were included were *Xylocopa* sp. 1 (2%), *Xylocopa* sp. 2 (0.9%), *Ceratina* sp. 1 (2.1%), *Ceratina* sp. 2 (1.5%), *Nomia* sp.1 (13.5%), *Nomia* sp. 2 (19%), *Halictus* sp. (6%), *Anthopora* sp., and *Lasioglossum* sp. (8%) (Figure 1). While sampling, we also observed some visits from *Apis dorsata,* however these were negligible in number and moreover, the individuals did not enter *S. melongena* flowers, hence the species was excluded from our analyses.

3.2 *Solanum melongena* Pollen

Most of the pollen grains of *S. melongena* were oblate spheroid, with a poroid aperture and an echinate pollen wall (Figure 2). However, monocolpate, tricolpate, and colpate shaped pollen apertures were also observed. The pollen grains were found to be 24.4 ± 0.06 µm in diameter. The pollen aperture size was 4.82 ± 0.05 µm and the distance between the exine-intine was 2.058 ± 0.004 µm.

3.3 Pollen Load Analyses

Of the 11 species of bees observed pollinating *S. melongena*, we were able to collect pollen loads from 10 individuals each of 7 species: *Apis florae*, *Apis cerana*, *Nomia* sp.1, *Nomia* sp. 2, *Halictus* sp., *Xylocopa* sp.1, and *Lassioglossum* sp. Most of the bees were observed exhibiting buzz (vibration) pollination behaviour on *S. melongena* flowers.

There were significant differences in *S. melongena* pollen load size among the bee species ($H = 13.45$, $P < 0.001$). The average pollen load ranged from a few thousand pollen grains to more than 134,000 grains per individual in some species. *Xylocopa* sp. 1 individuals had highest number of pollen grains on their bodies followed by *A. cerana* and *Nomia* sp.1. The smaller sweat bees, *Lasioglossum* sp., had a lower pollen carrying capacity and hence a smaller pollen load as compared to other bee species (Table 1).

Solanum melongena pollen constituted over 80% of the pollen load for all the bee species except *Lasioglossum* sp. (Table 1). The mean proportions of conspecific and heterospecific pollen found in pollen load and the PPI varied from 0 to 100 indicating the variation in the bees' diets (Table 2). Some bee species had more floral species as sources of pollen than others.

3.4 Pollen Protein Analyses

The protein content of mature *S. melongena* pollen was found to be 39.3 ± 1.6 mg proteins/g of pollen. Peptides were identified by the pollen protein chromatogram obtained by MS spectra (Figure 3). Peptides present in the pollen have a molecular weight of 18.1kDa to 157kDa, with a pI range from 5.27 to 9.25 (Table 3). Functions of major proteins could be identified based on previous literature (Table 4). Ninety percent of the proteins could be grouped as either energy production proteins, defence proteins, or protein synthesizing and processing proteins. The remainder consisted of superoxide dismutase (SOD), RNA2 polyprotein, DNA-directed RNA polymerase subunit beta (β), NADH-quinone oxidoreductase subunit H, NBS-LRR resistance protein, xyloglucan specific endoglucanase inhibitor, L-galactose dehydrogenase, chloroplast polyphenol oxidase, DNA-directed RNA polymerase subunit beta.

Discussion

The results indicate bee species can have their nutritional needs met in *Solanum melongena* agroecosystems. This is highly desirable for both wild bee species as well as the vegetable crop. *Solanum melongena* pollen are foraged by a wide range of bee species indicating that they are a significant food source to a wide range of bee diets. Because *S. melongena* flowers through the year, it provides pollen on a consistent basis for bee species. Many of the bee species sampled in this study have also been observed agroecosystems in northern parts of India [24, 25]. Apidae and Halictidae, in particular include a diverse set of species that often have locally abundant populations [17].

All the species sampled, except *Lasioglossum,* had pollen loads that consisted of over 75% *S. melongena* pollen. Pollination of *S. melongena* seems to be mainly carried out by the carpenter bee *Xylocopa*, the dwarf bee *Apis florae,* and *Nomia* sps**.** Even in *Lasioglossum*, *S. melongena* comprised most of the pollen load; about 53%. The genus *Lasioglossum* (the sweat bees) consists of generalists and even though they might have a comparatively lower proportion of conspecific pollen on their bodies, they have been shown to have a higher pollen deposition rate on crops such as watermelon as compared to the managed honey species *A. mellifera* [26]. This may also be the case in *S. melongena* though further studies are needed to confirm the same.

Plant species that are obligate insect-pollinated such as buzz pollinated taxa have protein rich sources [12]. *Solanum melongena* pollen has a significant number of proteins. Additionally, many of the proteins and their constituent amino acids are needed in energy production, defence, or protein synthesis. Studies on *Arabidopsis thaliana* found that half of the identified proteins are involved in metabolism (20%), energy generation (17%), or cell structure (12%) ([27]). Similar results have also been reported in tomato (*Lycopersicon esculentum*) pollen [28]. Our results are in keeping with the literature. In *S. melongena*, defence related proteins such as superoxide dismutase are the first line of defence in pollen stress for free floating pollen grains prior to pollination. Energy related proteins such as β-galactosidase, NADH-quinone oxidoreductase subunit H, and L-galactose dehydrogenase serve as a nutritional reward for pollinators. Protein synthesizing and processing proteins such as (β) and DNA-directed RNA polymerase subunit beta' (Acc. No. A0A165BB56) participate in cellular functions or building proteins.

Both amount and quality of protein are important to wild bees; poor pollen protein implies that a bee will more foraging trips to collect high quantity pollen [29]. Having good protein quality such as in *S. melongena* means that the foraging bees will have to make fewer trips. There are reports that in social bees a high protein diet is associated with enhanced fecundity rates and better chance of survival through diapause [30]. It is also important to examine the presence and impact of secondary compounds such as alkaloids on wild bees. *Solanum melongena* is a member of the family Solanaceae, known for its rich

diversity of alkaloids. There is very little information on the presence of alkaloids in pollen or the toxic effects of these secondary compounds on wild bees.

Our study demonstrates the biochemical and nutritive composition of *S. melongena* mature pollen and its potential to conserve wild bees by playing a significant role in their diets. Further studies should examine the impact of stressors such as insecticide and pesticide residue on pollen. Honey bees with chronic exposure to thiamethoxam, a neonictotinoid, were negatively impacted during larval development even with high quality pollen, though the effect was considerably more with lower quality pollen [31]. Future studies should also examine the impact of the landscape surrounding the crop field which have been found to influence pollinator diversity [32]. More studies are needed in mixed agricultural systems that have more than one crop and hence a variation in the nutritive content of the pollen from an agroecosystem.

Conclusion

We conclude that *Solanum melongena* can play a significant role in the nutrition and diet to a variety of wild bee species in agroecosystems by providing substantial protein content in the pollen. Moreover, many of the proteins are important in the cellular, metabolic, and defence functions of the bee species.

Acknowledgements

The authors are grateful to authorities of National Agricultural Research Project, MPKV, Rahuri, Maharashtra, India for permitting sample collection in their fields. The authors thank Savitribai Phule University, Pune for the research grant funding sanctioned under DST PURSE and DRDP schemes.

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contribution

Authors Ramnath Andhale, Kalpana Pai, RS Pandit and Aswini Pai designed the study and contributed to field work, analyses, and writing the manuscript. Authors Vishal Pathare and Matthew Skeels contributed to conducting the biochemistry methods and interpreting the biochemistry data.

References

1. Klein AM, Vaissière BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, Tscharntke T. Importance of pollinators in changing landscapes for world crops. Proc R Soc B 2007;274(66)95–96.
2. Willmer PG, Cunnold H, Ballantyne G. Insights from measuring pollen deposition: quantifying the pre-eminence of bees as flower visitors and effective pollinators. Arthropod-Plant Inte. 2017;11(3),411-425.
3. Chittka L, Raine NE. Recognition of flowers by pollinators. Curr Opin Plant Biol. 2006;9(4):428-435.
4. Dyer AG, Paulk AC, Reser DH. Colour processing in complex environments : insights from the visual system of bees. Proc. R. Soc. B. 2011;278(1707), 952-959.
5. Vanderplanck M, Moerman R, Rasmont P, Lognay G, Wathelet B, Wattiez R, et al. How Does Pollen Chemistry Impact Development and Feeding Behaviour of Polylectic Bees? PLoS ONE. (2014) 9(1): e86209. Available https://doi.org/10.1371/journal.pone.0086209. Accessed 30 November 2022.
6. Russo L, Vaudo AD, Fisher CJ, Grozinger CM, Shea K. 2019. Bee community preference for an invasive thistle associated with higher pollen protein content. Oecologia. 2019;190(2):901-912
7. Roulston TH, Cane JH. The effect of pollen protein concentration on body size in the sweat bee *Lasioglossum zephyrum* (Hymenoptera:Apiformes). 2002;Evol Ecol 16(1):49–65.
8. Quinlan G, Milbrath M, Otto C, Smart A, Iwanowicz D, Cornman RS, et al. Honey bee foraged pollen reveals temporal changes in pollen protein content and changes in forager choice for abundant versus high protein flowers. Agr Ecosyst Env. 2021;322(107645). Available https://www.sciencedirect.com/ science/article/pii/S0167880921003492. Accessed November 30 2022.
9. Baloglu GH, Gurel F. The effects of pollen protein content on colony development of the Bumblebee, *Bombus terrestris* L. J. Apic. Sci. 2015;59(1):83-88.
10. Moerman R, Vanderplanck M, Fournier D, Jacquemart A, 2017. Pollen nutrients better explain bumblebee colony development than pollen diversity. R Entom Soc 2017;10(2):171–179.
11. Leach ME, Drummond F. A review of native wild bee nutritional health. Int. J. Ecol. 2018;Article ID 9607246, 10 pages. Available: https://doi.org/10.1155/2018/9607246. Accessed 30 November 2022.
12. Hanley ME, Franco M, Pichon S, Darvill B, Goulson D. Breeding system, pollinator choice and variation in pollen quality in British herbaceous plants. Funct. Ecol. 2008;22(4):592-598.
13. Jankauski M, Ferguson R, Russell A, Buchmann S. Structural dynamics of real and modelled *Solanum* stamens: implications for pollen ejection by buzzing bees. J R Soc Interface. 2022;19:188. Available: https://royalsocietypublishing.org/doi/10.1098/rsif.2022.0040 Accessed 30 November 2022.
14. Tavares PRA, Alves Jr. VV, Morais GA, Polatto LP, Dutra JCS. Pollen availability and behavior of visiting bees of Solanum lycocarpum A. St. Hill (Solanaceae). Entomol. News. 2018;127(4):375–385.
15. Government of India. Horticultural statistics at a glance 2018. Ministry of Agriculture and Farmer's Welfare, Department of Agriculture, Cooperation and Farmers' Welfare Horticulture Statistics division. Available: https://agricoop.nic.in/Documents/Horticulture%20Statistics%20at%20a%20Glance-2018.pdf. Accessed 30 November 2022.
16. Shanika UJM, Jayasinghe R, Saumya TH, Silva E, Inoka WA, Karunaratne P. Buzzing wild bee visits enhance seed set in eggplant, Solanum melongena. Psyche: J. Entom. 2017. Available https://doi.org/10.1155/2017/4624062. Accessed 30 November 2022.
17. Michener CD. The bees of the world. 2nd ed. Baltimore: John Hopkins university press. 2007
18. Faegri K, Kaland P E, Krzywinski K. Textbook of pollen analysis. 4th ed. John Wiley and Sons Ltd., Chichester, UK. 1989
19. Ne'eman G, Dafni A, Potts SG. A new pollination probability index (PPI) for pollen load analysis as a measure for pollination effectiveness of bees. J Apic Res 1999:38(1-2):19-23.
20. Hammer Ø, Harper DAT, Ryan PD. 2001. PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4(1). http://palaeo-electronica.org/2001_1/past/issue1_01.htm. Accessed 30 November 2022.
21. Wu X, Xiong E, Wang W, Scali M, Cresti M. Universal sample preparation method integrating trichloroacetic acid/acetone precipitation with phenol extraction for crop proteomic analysis. Nat Protoc. 2014;9(2):362-374.
22. Bradford, MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing principle of protein-dye binding. Anal. Biochem. 1976;72(1-2):248–254.

23. Szczęsna T. Protein content and amino acid composition of bee-collected pollen from selected botanical origins. J Apic Sci. 2006;50(2):81–90.
24. Batra SWT. Crop pollination and the flower relationships of the wild bees of Ludhiana , India. J. Kans Entomol Soc. 1967;40(2):164–177.
25. Abrol DP, Shankar U, Chatterjee D., Ramamurthy VV. Exploratory studies on diversity of bees with special emphasis on non-Apis pollinators in some natural and agricultural plants of Jammu division, India. Curr Sci. 2012;103(7):780-783.
26. Njoroge GN, Gemmill B, Bussmann R, Newton LE, Ngumi VM. Diversity and efficiency of wild pollinators of watermelon (Citrullus lanatus (Thunb.) Mansf.) at Yatta (Kenya). J Appl Hortic. 2010;12(1):35–41.
27. Holmes-Davis R, Tanaka CK, Vensel WH, Hurkman WJ, McCormick S. Proteome Mapping of mature pollen of *Arabidopsis thaliana.* Proteomics. 2005;18(5):4864-4884.
28. Sheoran IS, Ross AR, Olson DJ, Sawhney VK. Proteomic analysis of tomato (*Lycopersicon esculentum*) pollen. J Exp Bot. 2007;58(13):3525–3535.
29. Neff J, Simpson B. Nesting and foraging behavior of *Andrena* (*Callandrena*) *rudbeckiae* Robertson (Hymenoptera: Apoidea: Andrenidae) in Texas. J Kansas Entom Soc 1997;70(2):100–113.
30. Kim, JY. Female size and fitness in the leaf-cutter bee Megachile apicalis. Ecol Entomol 1997;22(3):275–282.
31. Renzi MT, Rodríguez-Gasol N, Medrzycki P, Porrini C, Martini A, Burgio G, et al. Combined effect of pollen quality and thiamethoxam on hypopharyngeal gland development and protein content in *Apis mellifera*. Apidologie 2016; 47:779–788.
32. Patrício-Roberto GB, Campos MJO. Aspects of landscape and pollinators-what is important to bee conservation? Diversity. 2014;6(1):158–175.
33. Dai S, Li L, Chen T, Chong K, Xue Y, Wang T. Proteomic analyses of Oryza sativa mature pollen reveal novel proteins associated with pollen germination and tube growth. Proteomics. 2006 Apr;6(8):2504-2529.
34. Noir S, Bräutigam A, Colby T, Schmidt J, Panstruga R. A reference map of the Arabidopsis thaliana mature pollen proteome. Biochem Biophys Res Commun. 2005 Dec 2;337(4):1257-66.

Table 1: Mean counts and proportion of conspecific (PCP) *Solanum melongena* pollen grains collected from pollen loads of individual wild bee species in agricultural areas near Pune, Maharashtra, India. PBP is the proportion of bees out of the total sampled bees carrying *S. melongena* pollen, PPI represents the pollination probability index for each bee species.

Bee species	Mean (± S.E.) pollen grains per pollen load	PCP	PBP	PPI
Apis cerana	32926 ± 422.4	0.859	0.141	0.121
Apis florea	12926 ± 745.1	0.841	0.159	0.133
Halictus sp.	18780.49 ± 243.9	0.935	0.065	0.061
Lassioglosum sp.	3658.5 ± 505.7	0.533	0.467	0.249
Nomia sp. 1	24959 ± 722.6	0.961	0.039	0.038
Nomia sp.2	10731 ± 243.9	0.864	0.136	0.118
Xylocopa sp.	134146.3 ± 6813.2	0.902	0.098	0.088

Table 2: Proportion of identified proteins and their possible function in *Solanum melongena* pollen collected from agricultural areas near Pune, Maharashtra, India.

Accession No.	Name of protein and source	Protein content	Protein function	Reference
P00722	Beta-galactosidase OS=Escherichia coli (strain K12) GN=lac Z PE=1 SV=2 - [BGAL_ECOLI]	30 %	Energy production	[28]
A0A168RDF6	NADH-quinone oxidoreductase subunit H OS=Solanum melongena GN=ndh A PE=3 SV=1 - [A0A168RDF6_SOLME]			
A0A1L2JIV8	L-galactose dehydrogenase OS=Solanum melongena GN=GalDH PE=2 SV=1 - [A0A1L2JIV8_SOLME]			
A1XIQ1	RNA2 polyprotein OS=Tomato torrado virus (isolate Solanum lycopersicum/Spain/PRIToTV0301/-) PE=3 SV=1 - [POL2_TOTV]	50 %	Défense	[28]
A0A1L6Z9M5	Superoxide dismutase [Cu-Zn] OS=Solanum melongena PE=2 SV=1 - [A0A1L6Z9M5_SOLME]			
D6QUQ0	Xyloglucan specific endoglucanase inhibitor OS=Solanum melongena PE=2 SV=1 - [D6QUQ0_SOLME]			
A0A060AJG6	NBS-LRR resistance protein (Fragment) OS=Solanum melongena GN=RGA5 PE=4 SV=1 - [A0A060AJG6_SOLME]			
A0A165BB56	DNA-directed RNA polymerase subunit beta" OS=Solanum melongena GN=rpoC2 PE=3 SV=1 - [A0A165BB56_SOLME]	10 %	Protein synthesis and processing	[33, 34]
A0A160I962	DNA-directed RNA polymerase subunit beta OS=Solanum melongena GN=rpoB PE=3 SV=1 - [A0A160I962_SOLME]			
F1DBB7	Chloroplast polyphenol oxidase (fragment) OS=Solanum melongena GN=PPO4 PE=2 SV=1 - [F1DBB7_SOLME]			

Table 3: List of proteins (identified using LC-MS and MALDI- TOF) in *Solanum melongena* pollen collected from agricultural areas near Pune, Maharashtra, India. All peptides corresponded with the peptides that were above the threshold in our Proteome Discoverer search (expected $p < 0.05$).

Accession no.	Name of Peptides	No. of AAs	MW [kDa]
A0A1L6Z9M5	Superoxide dismutase [Cu-Zn] OS=Solanum melongena PE=2 SV=1 - [A0A1L6Z9M5_SOLME]	152	15.2
A1XIQ1	RNA2 polyprotein OS=Tomato torrado virus (isolate Solanum lycopersicum/ Spain/ PRIToTV0301) PE=3 SV=1 - [POL2_TOTV]	1198	133.6
A0A160I962	DNA-directed RNA polymerase subunit beta OS=Solanum melongena GN=rpoB PE=3 SV=1 - [A0A160I962_SOLME]	1070	120.6
A0A168RDF6	NADH-quinone oxidoreductase subunit H OS=Solanum melongena GN=ndhA PE=3 SV=1 - [A0A168RDF6_SOLME]	363	40.1
A0A060AJG6	NBS-LRR resistance protein (Fragment) OS=Solanum melongena GN=RGA5 PE=4 SV=1 - [A0A060AJG6_SOLME]	158	18.1
D6QUQ0	Xyloglucan specific endoglucanase inhibitor OS=Solanum melongena PE=2 SV=1 - [D6QUQ0_SOLME]	437	46.7
A0A1L2JIV8	L-galactose dehydrogenase OS=Solanum melongena GN=GalDH PE=2 SV=1 - [A0A1L2JIV8_SOLME]	321	34.6
F1DBB7	Chloroplast polyphenol oxidase (Fragment) OS=Solanum melongena GN=PPO4 PE=2 SV=1 - [F1DBB7_SOLME]	584	66.2
A0A165BB56	DNA-directed RNA polymerase subunit beta'' OS=Solanum melongena GN=rpoC2 PE=3 SV=1 - [A0A165BB56_SOLME]	1392	157.1

Table 4: List of amino acids and associated nutritive value in proteins of *Solanum melongena* pollen collected from agricultural areas near Pune, Maharashtra, India.

Name of Protein	Accession No.	Total Amino Acids	Alanine	Aspartic Acid	Glutamic Acid	Glycine	Leucine	Serine	Valine	%nutritive amino acid	Nutritive Value
Beta-galactosidase	P00722	1024	77	64	62	71	96	60	64	48.242	494
Superoxide dismutase	A0A1L6Z9M5	152	13	10	7	27	10	11	9	57.237	87
RNA2 polyprotein	A1XIQ1	1198	88	47	73	76	103	107	86	48.414	580
NADH-quinone oxidoreductase subunit H	A0A168RDF6	367	19	8	18	31	63	35	21	53.133	195
NBS-LRR resistance protein	A0A060AJG6	158	11	14	8	9	20	9	7	49.367	78
Xyloglucan specific endoglucanase inhibitor	D6QUQ0	437	30	15	9	36	40	46	35	48.284	211
L-galactose dehydrogenase	A0A1L2JIV8	321	28	17	20	28	37	26	23	55.763	179
Chloroplast polyphenol oxidase	F1DBB7	584	35	42	28	36	47	34	29	42.979	251
DNA-directed RNA polymerase subunit beta GN=rpoB	A0A160I962	1070	54	45	76	91	111	74	62	47.944	513
DNA-directed RNA polymerase subunit β GN=rpoC	A0A165BB56	1392	54	59	73	99	128	115	89	44.325	617

List of Figures and their Captions:

Figure 1: Species of wild bees visiting *Solanum melongena* flowers and their relative abundance in agricultural fields in Pune, Mahrashtra, India.

Figure 2: Images of *Solanum melongena* pollen under (A) a Scanning Electron Microscope (FESEM, FEI Nova SEM™ 450) and (B) a light microscope (Zeiss AX10) at 40 x magnification.

Figure 3: Chromatogram of protien extract of *Solanum melongena* pollen indicating realtive abundances of various protiens.

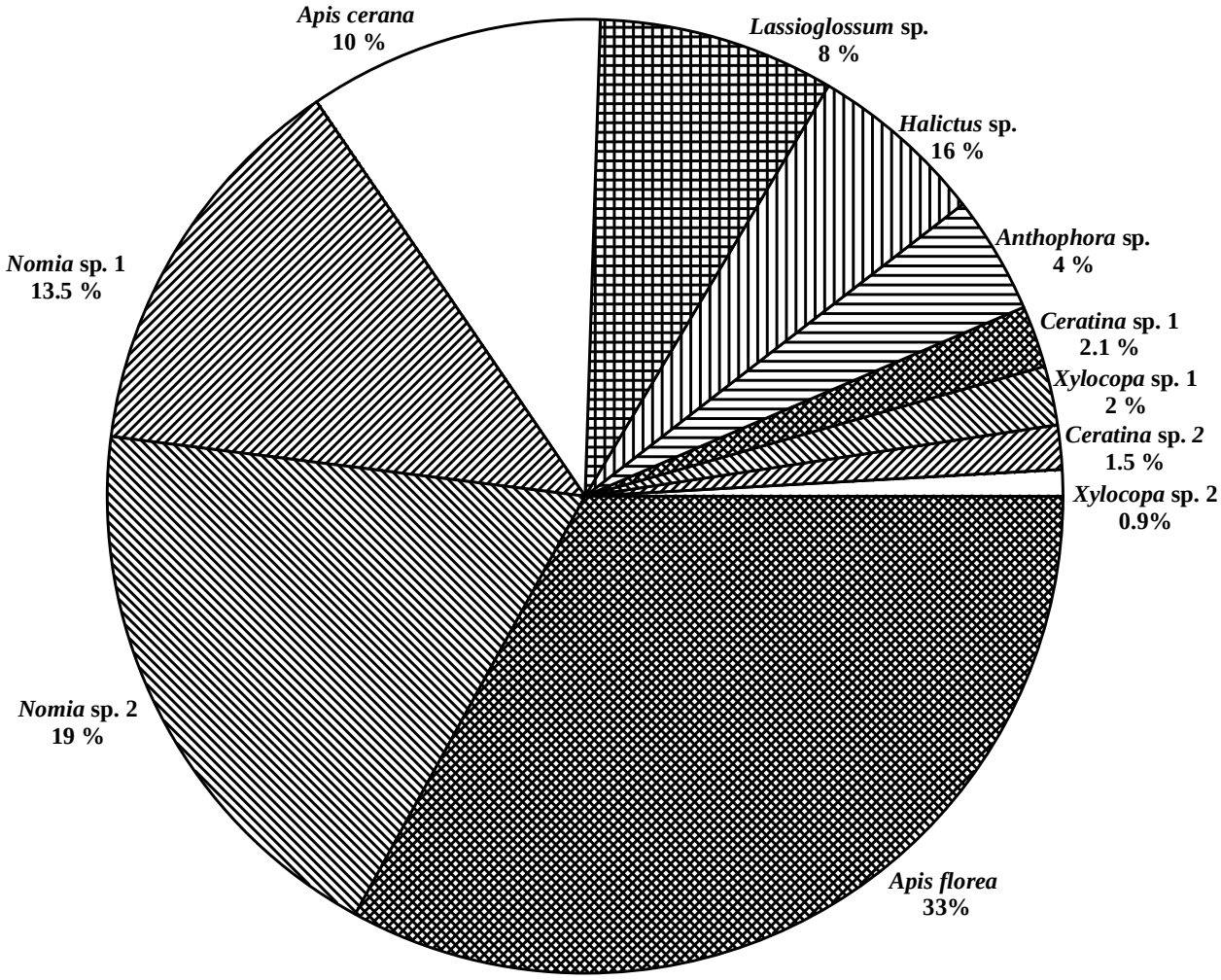
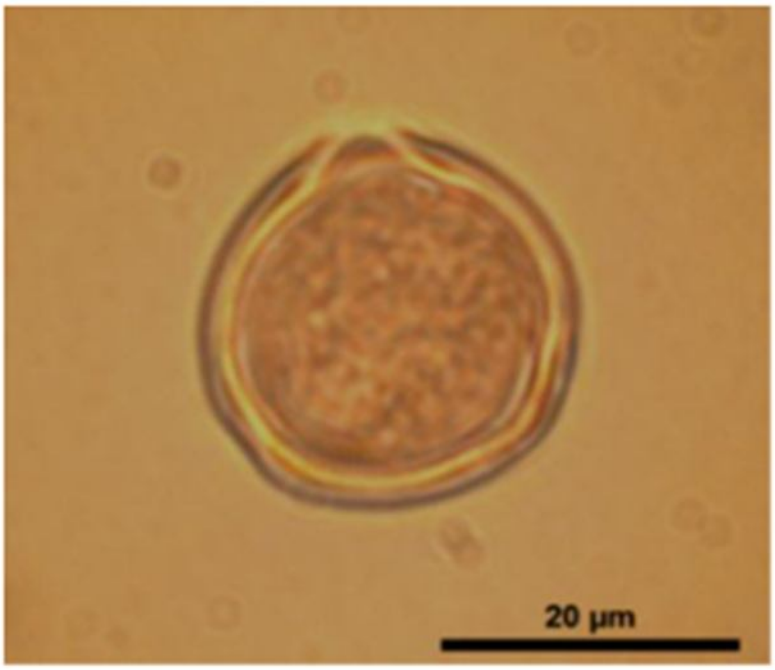
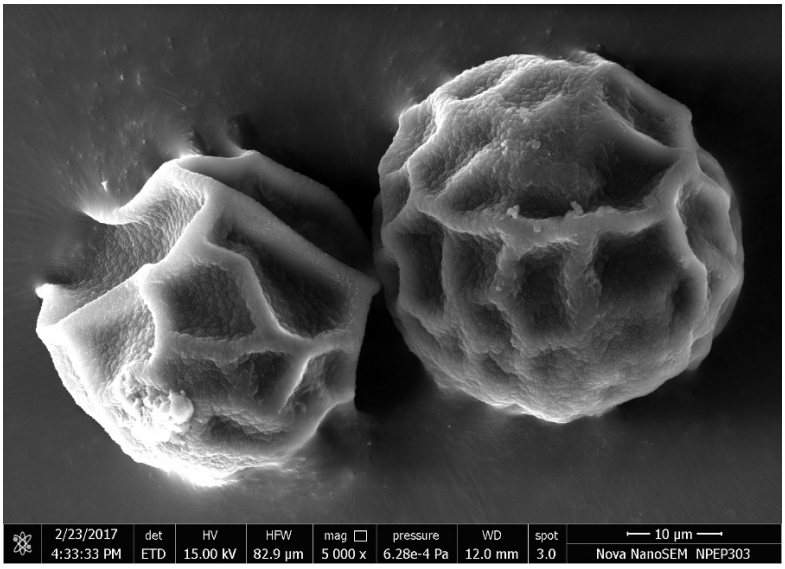

Apis cerana
10 %
Lassioglossum sp.
8 %
Halictus sp.
16 %
Anthophora sp.
4 %
Ceratina sp. 1
2.1 %
Xylocopa sp. 1
2 %
Ceratina sp. 2
1.5 %
Xylocopa sp. 2
0.9%
Apis florea
33%
Nomia sp. 2
19 %
Nomia sp. 1
13.5 %

Figure 1


20 µm

A


2/23/2017
4:33:33 PM
det
ETD
HV
15.00 kV
HFW
82.9 µm
mag
5 000 x
pressure
6.28e-4 Pa
WD
12.0 mm
spot
3.0
10 µm
Nova NanoSEM NPEP303

B

Figure 2

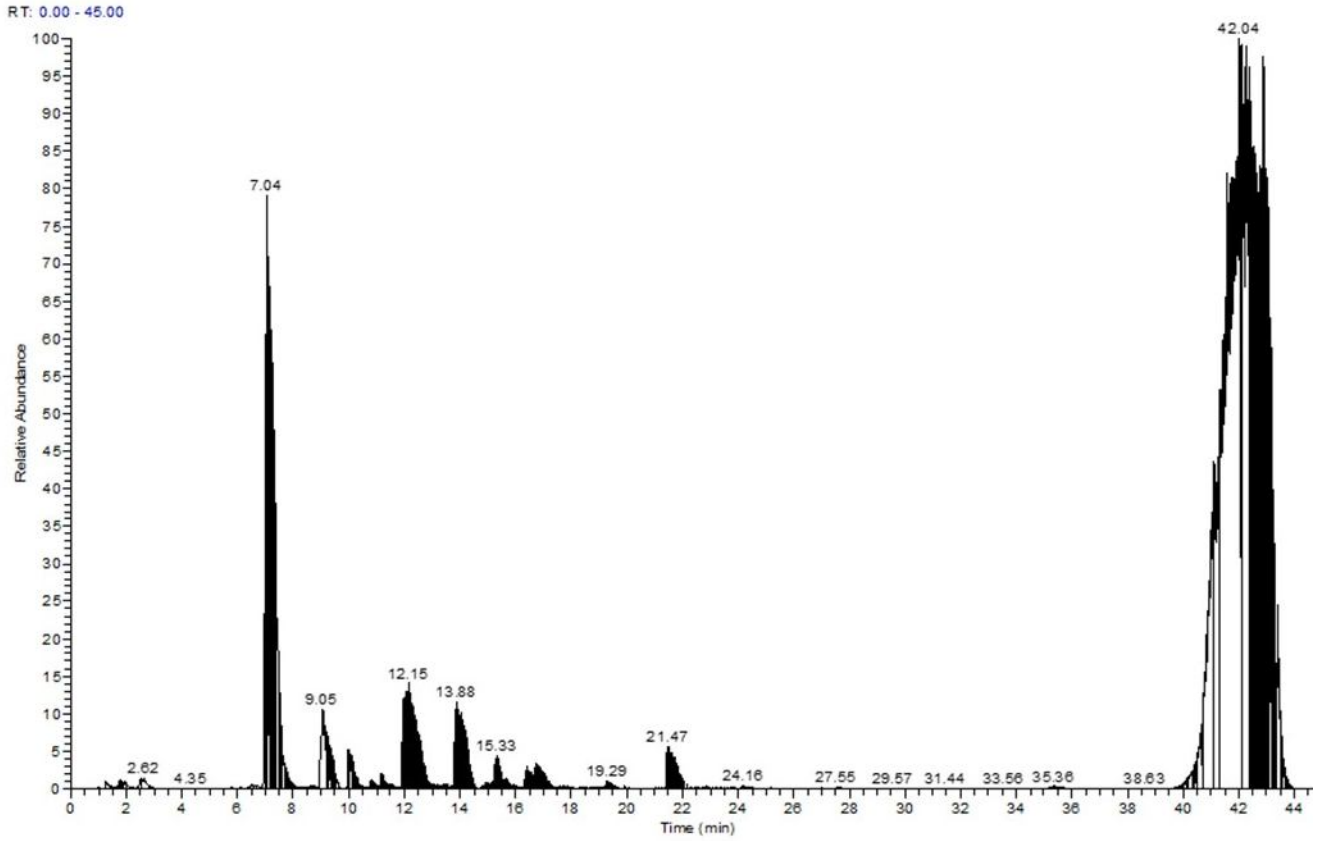

RT: 0.00 - 45.00
Relative Abundance
Time (min)
7.04
42.04
12.15
13.88
9.05
21.47
15.33
2.62
4.35
19.29
24.16
27.55
29.57
31.44
33.56
35.36
38.63

Figure 3