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Composition and Nutrition of Wild Bees in Solanum melongena L. Agroecosystems in Pune, Maharashtra, India

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Authors' contributions

This work was carried out in collaboration among all authors. Authors RA, KP, RSP and AP designed the study and contributed to field work, analyses, and writing the manuscript. Authors VP and MS contributed to conducting the biochemistry methods and interpreting the biochemistry data. All authors read and approved the final manuscript.

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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

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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 rearing, survival. brood size and 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 includina tomatoes species (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. MATERIALS 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 [17]. 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 SEMTM 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: $PPI = PCP \times PBP$ 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 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 60° 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/ul and incubated for 4 hours at 37° 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 DiscovererTM (Thermofisher Scientific), operated on a local server, was used to search the NCBI database (Fig. 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%) (Fig. 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 (Fig. 2). However, monocolpate, tricolpate, and colpate shaped pollen apertures were also observed. The pollen grains were found to be $24.4 \pm 0.06 \ \mu m$ in diameter. The pollen aperture size was $4.82 \pm 0.05 \ \mu m$ and the distance between the exine-intine was $2.058 \pm 0.004 \ \mu m$.

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).

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= <i>Solanum melongena</i> GN=ndh A PE=3 SV=1 - [A0A168RDF6_SOLME]			
A0A1L2JIV8	L-galactose dehydrogenase OS= <i>Solanum melongena</i> 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= <i>Solanum melongena</i> PE=2 SV=1 - [A0A1L6Z9M5_SOLME]			
D6QUQ0	Xyloglucan specific endoglucanase inhibitor OS= <i>Solanum melongena</i> PE=2 SV=1 - [D6QUQ0_SOLME]			
A0A060AJG6	NBS-LRR resistance protein (Fragment) OS= <i>Solanum melongena</i> GN=RGA5 PE=4 SV=1 - [A0A060AJG6_SOLME]			
A0A165BB56	DNA-directed RNA polymerase subunit beta" OS= <i>Solanum melongena</i> GN=rpoC2 PE=3 SV=1 - [A0A165BB56_SOLME]	10%	Protein synthesis and processing	[33,34]
A0A160I962	DNA-directed RNA polymerase subunit beta OS= <i>Solanum melongena</i> GN=rpoB PE=3 SV=1 - [A0A160I962_SOLME]			
F1DBB7	Chloroplast polyphenol oxidase (fragment) OS= <i>Solanum melongena</i> 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

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= <i>Solanum melongena</i> GN=ndhA PE=3 SV=1 - [A0A168RDF6_SOLME]	363	40.1
A0A060AJG6	NBS-LRR resistance protein (Fragment) OS= <i>Solanum melongena</i> GN=RGA5 PE=4 SV=1 - [A0A060AJG6_SOLME]	158	18.1
D6QUQ0	Xyloglucan specific endoglucanase inhibitor OS= <i>Solanum melongena</i> 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

All peptides corresponded with the peptides that were above the threshold in our Proteome Discoverer search (expected p < 0.05)

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

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

 Maharashtra, India

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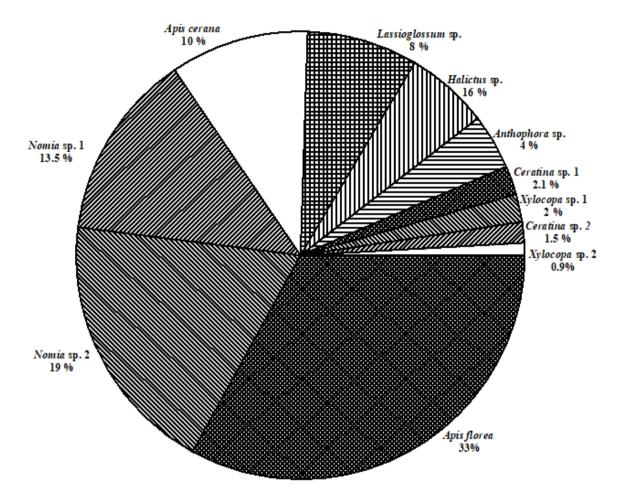
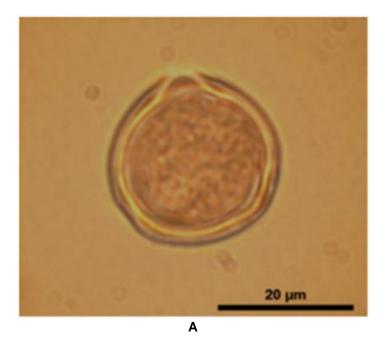


Fig. 1. Species of wild bees visiting *Solanum melongena* flowers and their relative abundance in agricultural fields in Pune, Mahrashtra, India



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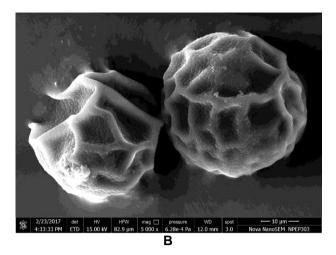


Fig. 2. Images of *Solanum melongena* pollen under (A) a Scanning Electron Microscope (FESEM, FEI Nova SEM[™] 450) and (B) a light microscope (Zeiss AX10) at 40 x magnification

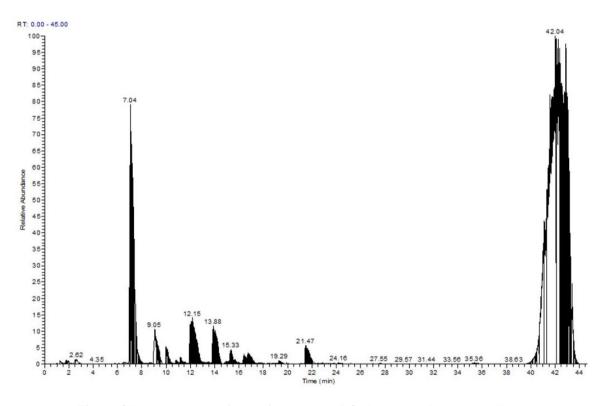


Fig. 3. Chromatogram of protein extract of *Solanum melongena* pollen indicating relative abundances of various proteins

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 (Fig. 3). Peptides present in the pollen have a molecular weight of 18.1kDa to 157kDa, with a pl 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.

4. 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 βgalactosidase, 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.

5. 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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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