Pollen analysis of *Apis cerana indica* F. winter honeys from Sub-Himalayan West Bengal, India

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Qualitative and quantitative analysis of seven squeezed honey samples collected during November '1996- February '1997 from seven different rural areas of sub-Himalayan West Bengal were made with a view to determine the pollen frequencies and also to ascertain the composition of honey in the area. Echhey Busty honey (EBH) and Samsing honey (SMH) were unifloral with *Aristolochia* sp. (52%) and *Rosa* sp. (78.5%) as predominant pollen types respectively. The remaining samples from Dungra Busty (DUNGH), Kurseong (KURH), Mongpu (MONGH), Seed Farm area, Kalimpong (SFH), Suruk (SURH) were multifloral. The significant nectar sources other than the predominent pollen types included *Brassica* sp., *Bidens pilosa, Erigeron karwinskianus, Tropaeolum majus, Camellia* sp., *Dahlia imperialis, Bellis perennis, Pimpinella* sp., *Trifolium repens, Ageratum conyzoides, Calendula officinalis, Cestrum* sp., *Dichroa febrifuga, Luffa* sp., *Solanum* sp., *Bauhinia* sp., *Clematis* sp. Group I honey (APC < 20000) and Group II honey (APC 20000-100000) are the characteristics of this area.

Key-words—*Apis cerana indica* F., Squeezed honey, Sub-Himalayan West Bengal.

INTRODUCTION

RECENT studies involving microscopic analysis of the pollen sectra of honeys from Sikkim and sub-Himalayan West Bengal facilitated recognition of honey samples from various floristic and geographical regimes (Ganguly *et al.*, 1984; Bera *et al.*, 1997). These studies further indicate that the rich floral resources of this area can profitably be exploited by the beekeeping enterprises for enhanced and sustained honey production.

In the present work seven squeezed winter honey samples from sub Himalayan region of West Bengal (Fig. 1) are analysed to determine their botanical origin.

Previous investigations of honeys from the immediate environments of sub-Himalayan West Bengal suggest *Azadiracta indica, Blumea lacera, Coriandrum sativum, Trifolium repens, Clematis montana, Citrus reticulata, Brassica* sp., *Calendula officinalis, Rubus ellipticus, Torenia penduncularis, Centauria cyanus, Sechium edule, Clarkia pulchella, Moringa oleifera, Acacia auriculiformis* as the major source of pollen in honeys (Ganguly *et al.*, 1984; Bera *et al.*, 1997) during summer.

MATERIAL AND METHOD

From sub-Himalayan region of West Bengal 7 honey samples (6 from Darjeeling district and one from Jalpaiguri district) were analysed. These samples were collected during the period November, 1996 to February, 1997 (Table 1). The samples of *Apis cerana indica* F. were collected by squeezing the honey combs. Thus pure honey was obtained. Possibility of slight mixing of the honey with a few pollen loads from pollen store could not be ruled out. This could result in somewhat higher figures of absolute pollen count (APC). However, it would not alter qualitatively the totality of the picture of pollen type categories of each honey sample (Kalpana & Ramanujam, 1991).

300 pollen grains (100 per slide) were counted to determine the frequency classes. Four frequency classes were recognised as recommended by International Commission for Bee Botany (Louveaux *et al.*, 1978). These were predominant pollen types (> 45%), secondary pollen types (16-45%), important

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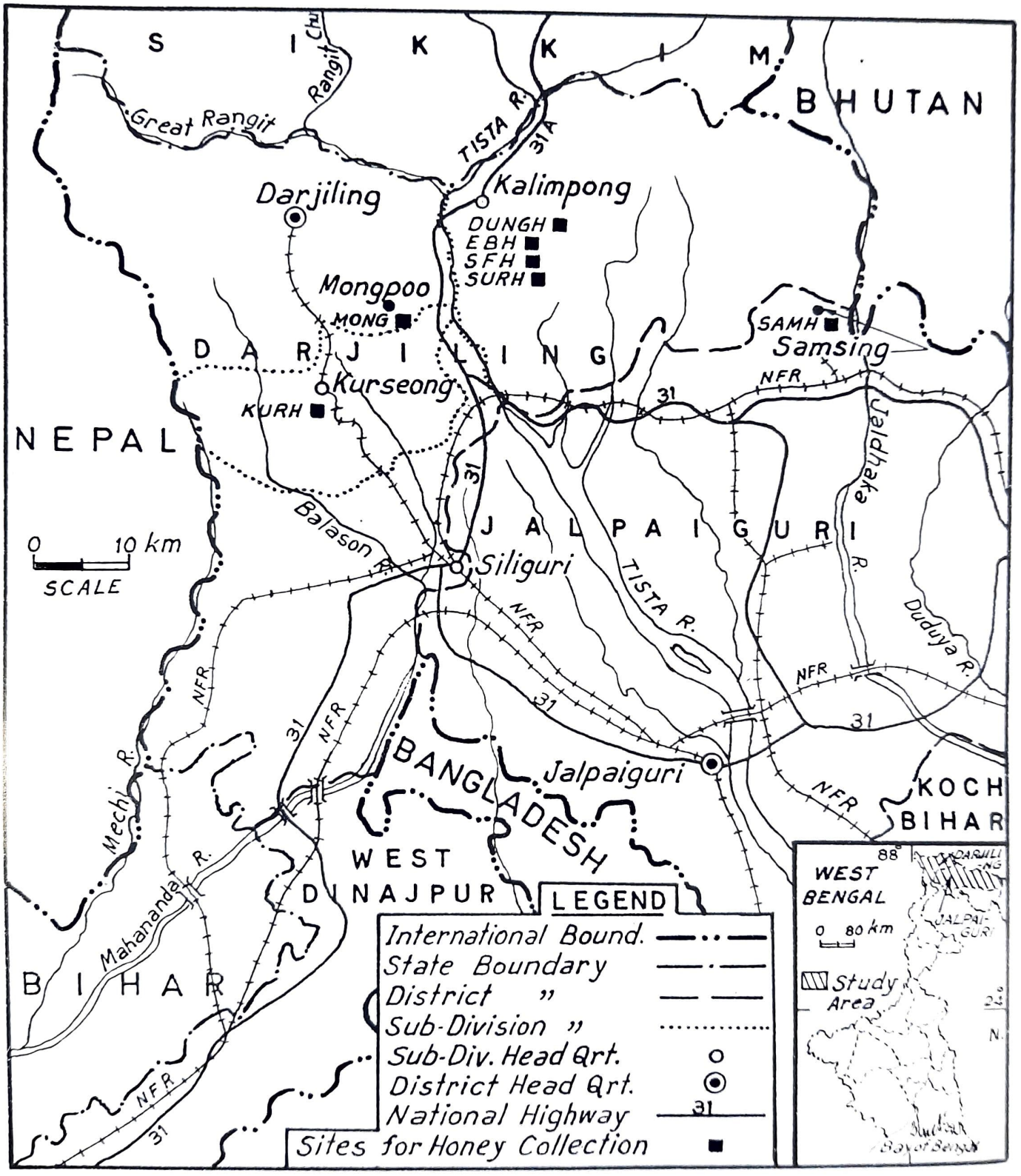

Location Map

minor pollen types (3-15%) and minor pollen types (<3%). The honeys with a predominant pollen type (with > 45% pollen of a single type) were termed unifloral and the rest multifloral.

Unacetolysed honey samples were examined for computing the ratio of honeydew elements (fungal spores and hyphae, algae and wax particles) and pollen grains of nectariferous plants (HDE/P). The absolute pollen counts (APC) of the honey samples (i.e. the number of pollen grains per 10 gm) were calcu-

Table 1. Inventory and pollen characterization of winter honeys

Honey sample	Period of collection	Colour	Altitude (m)	APC (Absolute pollen count /10 gm. of honey)	HDE/P	Group	Nature of honey	Frequency (%) of pollen types [upto important minor category (3%)]
DUNGH (Dungra Busty Honey, Kalimpong sub-division)	December, 1996	Amber	1152	1078	0.01	Gr.-I	Multifloral	*Brassica* sp. (36), *Bidens pilosa* (20), *Cardamine hirsuta* (18.5), *Macaranga* sp. (4.5), *Leucas* sp. (3.5), *Abelmoschus* sp. (10) *Coriandrum* sp. (4).
EBH (Echhey Busty Honey, Kalimpong sub-division)	January, 1997	Amber	1080	23377	0.009	Gr.-II	Unifloral	*Aristolochia* sp. (52), *Oenanthe thomsonii* (8.8), *Raphanus sativus* (4.5), *Buddleja asiatica* (4), *Tropaeolum majus* (5.9), *Brassica* sp. (5.6), *Spiraea* sp. (4.4), *Primula* sp. (4.6), *Oxalis* sp. (3.2), *Erigeron karwinskianus* (3.14).
KURH (Kurseong Honey)	November, 1996	Amber	144	5500	0.011	Gr.-I	Multifloral	*Tropaeolum majus* (25), *Nicotiana* sp. (25), *Streptosolen jamesonii* (12.5), *Dahlia imperialis* (12.5), *Camellia* sp. (12), *Majus surculosus* (11.2).
MONGH (Mongpu honey, Kalimpong, sub-division)	December, 1996	Light yellow	1300	926	0.01	Gr.-I	Multifloral	*Bellis perennis* (24.5), *Rosa* sp. (22.6), *Bidens pilosa* (9.32), *Pimpinella* sp. (11.32), *Tropaeolum majus* (7.54), *Trifolium repens* (3.7)
SAMH (Samsing Honey, Jalpaiguri district)	January, 1997	Yellow	275	1754	0.02	Gr.-I	Unifloral	*Rosa* spp. (78.5), *Ageratum conyzoides* (7.8)
SFH (Seed Farm Honey, Kalimpong sub-division)	February, 1997	Light yellow	1160	3382	0.008	Gr.-I	Multifloral	*Calendula officinalis* (32.45), *Brassica* sp. (24.03), *Nicotiana* sp. (5.04), *Luffa* sp. (5.1), *Dichroa febrifuga* (4.9), *Porana* sp. (4.9), *Spergula arvensis* (3.36), *Cestrum* sp. (3.5).
SURH (Suruk Honey, Kalimpong sub-division)	November, 1996	Yellow	490	2386	0.008	Gr.-I	Multifloral	Rosa spp. (24), *Brassica* sp. (22.2), *Clematis* sp. (20.0), *Bauhinia* sp. (10.0), *Solanum* sp. (7.5), *Holboelia latifolia* (7.0).

lated using haemocytometer (Suryanarayana *et al.*, 1981). The samples were categorised under various groups in conformity with the universally followed grading parameters viz. Group I (< 20000), Group II (20000-100000), Group III (100000-500000), Group IV (500000-1000000), Group V (> 1000000)(Louveaux *et al.*, 1978).

The frequency distribution (frequency of occurrence) of pollen types was determined from the total complement of honey samples and four discrete classes were recognised, namely (i) very frequent (Present in > 50% of the samples), (ii) Frequent (20-50%), (iii) Infrequent (10-20%) and (iv) Rare (< 10%) (Feller-Demalsy *et al.*, 1987).

The degree of similarity of honey samples collected more than once in number during the same period was measured by using a similarity index. It is calculated by the formula $2c/(a + b)$ where a and b represent the number of pollen types in each of the samples and c represents the number of pollen types common to all the samples taken in calculation (Kalpana & Ramanujam, 1989). The lower the similarity index, higher would be the diversity between the samples and vice-versa (Table 3).

Table 2. Frequency classes of pollen types

Pollen types	Honey Samples Codes						
	DUNGH	EBH	KURH	MONGH	SAMH	SFH	SURH
Acanthaceae							
Asystasia macrocarpa					I		
Justicia sp.	M						
Acanthus carduaceous						M	
Apiaceae							
Coriandrum sp.	M				M		
Pimpinella diversifolia				I			
Oenanthe thomsonii		I					
Aristolochiaceae							
Aristolochia spp.		P					
Asteraceae							
Ageratum conyzoides				S	I		
Bellis perennis				I			
Bidens pilosa	S						
Calendula oficinalis						S	
Centaurea sp.						M	
Dahlia sp.			I			M	
Erigeron karwinskianus		I		I			
Galinsoga parviflora				M			
Brassicaceae							
Cardamine hirsuta	S	M					
Brassica sp.	S	I		M	M	S	S
Raphanus sativus		I					
Buddlejaceae							
Buddleja asiatica		I					
Caesalpiniaceae							
Bauhinia sp.							I
Capparaceae							
Cleome sp.					M		
Convolvulaceae							
Porana racemosa						I	M
Cucurbitaceae							
Coccinea grandis							M
Luffa sp.				M		I	
Euphorbiaceae							
Croton sp.				M			
Macaranga sp.	I					M	
Fabaceae							
Trifolium repens				I			M
Desmodium sp.					M	M	M
Crotalaria sp.				M			
Flacourtiaceae							
Gynocardia odorata							M
Hydrangiaceae							
Dichroa febrifuga						I	
Lamiaceae							
Ocimum basilicum				M			
Plectranthus mollis						M	
Leucus sp.	I					M	

Liliaceae							
Allium sp					M		
Magnoliaceae							
Magnolia sp.						M	M
Michelia sp.					M	M	M
Malvaceae							
Abelmoschus sp.						M	
Moringaceae							
Moringa oleifera					M	M	
Oxalidaceae							
Oxalis sp.		I					
Primulaceae							
Primula sp.		I					
Ranunculaceae							
Clematis sp.			M				S
Rosaceae							
Rosa sp.		M		M	P	M	S
Prunus sp.						M	
Potentilla fulgens			M				
Spiraea sp.		I					
Rubiaceae							
Cinchona sp.				M			
Sapindaceae							
Cardiospermum helicacabum							M
Schisandraceae							
Schisandra sp.		M					
Scrophulariaceae							
Veronica sp.							M
Lindenbergia sp.					M	I	
Majus sulculosus			I				
Solanaceae							
Datura sp.				M			
Cestrum sp.						I	
Streptosolen jamesonii							
Solanum sp.			I				I
Nicotiana sp.			S			I	
Sonneratiaceae							
Duabanga sp.							I
Ternstroemiaceae							
Camellia sp.			I				
Tropaeolaceae							
Tropaeolum majus		I	S	I			
Verbenaceae							
Lantana camara						M	
Unidentified pollen type	M	M	M	M	M	M	M

P = Predominant pollen types (> 45%)
S = Secondary pollen types (16-45%)
I = Important minor pollen types (3-15%)
M = Minor pollen types (< 3%)

Table 3. Similarity indexes of honey samples studies

Honey samples	Total no. of pollen types	No. of pollen types common to both samples	Similarity Index
Unifloral- Unifloral (January)			
EBH (Echhey Busty, Kalimpong)	14	2 *e.g., Brassica, Rosa*	2 x 2/14+11=0.16
SAMH (Samsing, Jalpaiguri)	11		
Multifloral- Multifloral (Nov.-Dec.)			
KURH (Kurseong)	8	1 e.g. *Tropaeolum*	2x1/ 8 + 19 = 0.07
MONGH (Mongpu)	19		
Multifloral – Multifloral (Dec.- Feb)			
SFH (Seed farm, Kalimpong)	24	4 *e.g. Brassica, Abelmoschus, Justicia, Macaranga*	2x4/ 24+10= 0.12
DUNGH (Dungra Busty, Kalimpong)	10		
Unifloral- Multifloral (November-January)			
SURH (Suruk, Kalimpong sub-division)	12	3 e.g. *Rosa, Brassica, Desmodium*	2x3 /12+11=0.26
SAMH (Samsing, Jalpaiguri)	11		
Unifloral- Multifloral (December-January)			
EBH (Echhey Busty, Kalimpong)	14	2 e.g. *Cardomine, Brassica*	2x2 /14+10 =0.16
DUNGH (Dungra Busty, Kalimpong)	10		

OBSERVATION

Of the seven winter honey samples two were found to be unifloral and the rest five multifloral. Echhey Busty honey (BEH) from Kalimpong sub-division and Samsing honey (SAMH) from Jalpaiguri district were found to be unifloral with *Aristolochia* sp. (52%) and *Rosa* sp. (78.5%) as predominant pollen types respectively. The important minor pollen types up to 3% level in the above two unifloral samples were found to be *Oenanthe thomsonii* (8.8%), *Tropaeolum majus* (5.9%), *Brassica* sp (5.6%), *Buddleja asiatica* (4%) in Echhey Busty Sample (EBH) and *Ageratum conyzoides* (7.8%) in Samsing sample (SAMH) (Table 1). No secondary pollen type was recovered from these two samples.

In Dungra Busty honey (DUNGH) of Kalimpong

PLATE 1 ⟶

(All figures are magnified x 1000 unless otherwise mentioned)

1. *Clematis* sp.
2. *Dahlia imperialis*
3. *Cardiospermum halicacabum*
4. *Michelia* sp.
5. *Ocimum* sp.
6. *Calendula officinalis*
7. *Aristolochia* sp.
8. *Coccinea grandis*
9. *Brassica* sp.
10. *Rosa* sp.
11. *Oxalis* sp.
12. *Cleome* sp.
13. *Cestrum* sp.
14. *Camellia* sp. x500
15. *Tithonia diversifolia* x500
16. *Ageratum conyzoides*
17. *Bidens pilosa*
18. *Bauhinia* sp. x500

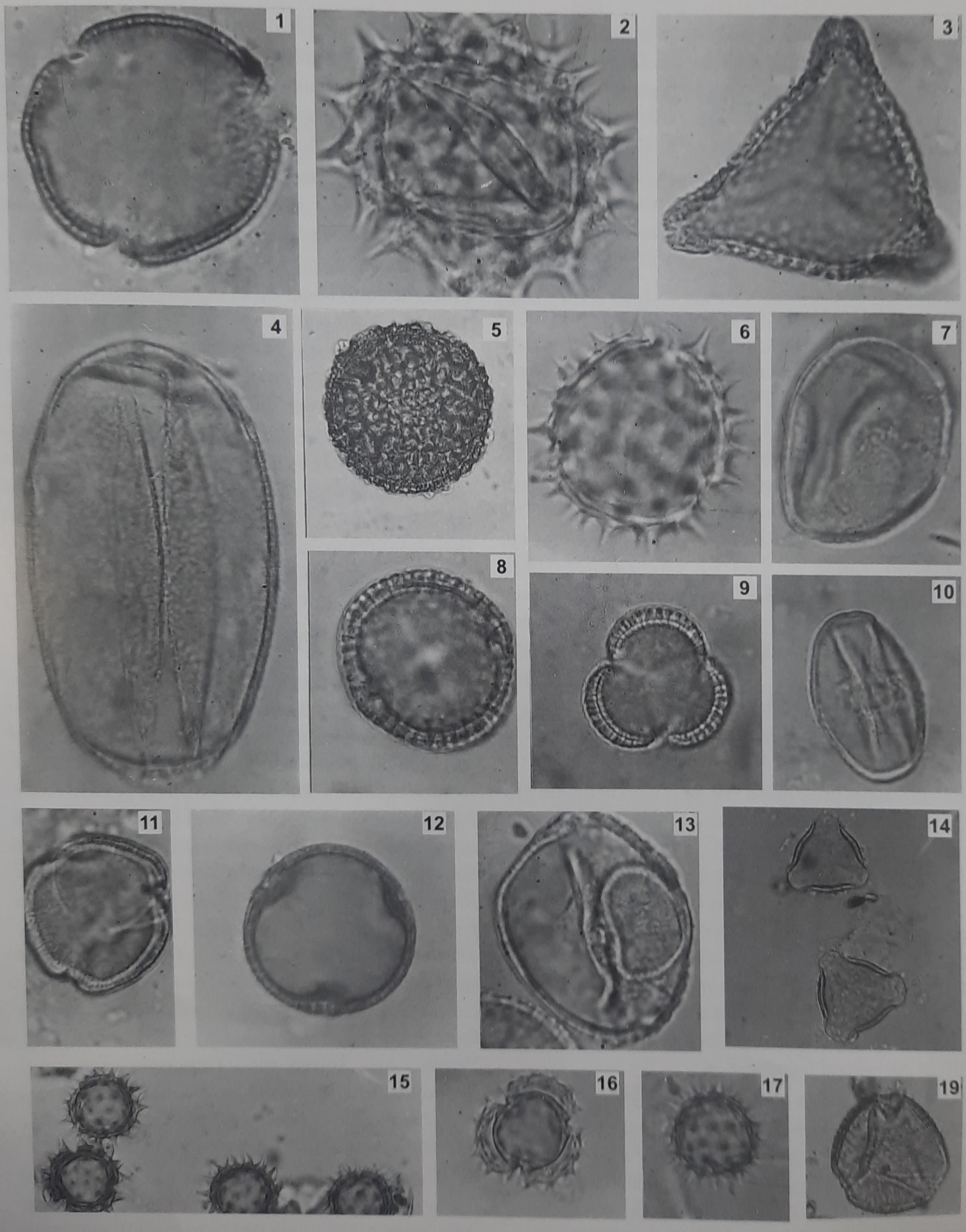

PLATE 1

Brassica sp. (36%), *Bidens pilosa* (20%) and *Cardamine hirsuta* (18.5%) were found to be secondary pollen types and other important minor pollen types recovered are shown in Table 1. The secondary pollen types recovered from Kurseong honey (KURH) were *Tropaeolum majus* (25%), *Nicotiana* sp. (25%). The important minor pollen types recovered from the same sample were *Streptosolen jamesonii* (12.5%), *Dahlia imperialis* (12.5%) and *Camellia* sp. (12.0%), etc. (Table 1). In Mongpu honey (MONGH) the secondary pollen types recovered were *Bellis perennis* (24.5%) and *Rosa* sp. (22.6%). The important minor pollen types were *Pimpinella* sp (11.32%), *Bidens pilosa* (9.32%), *Tropaeolum majus* (7.54%) and *Trifolium repens* (3.7%). In Seed Farm honey (SFH) of Kalimpong the secondary pollen types were found to be *Calendula officinalis* (32.45%) and *Brassica* sp. (24.03%). The important minor pollen types were *Nicotiana* sp. (5.04%), *Luffa* sp. (5.1%), *Dichroa febrifuga* (4.9%), *Porana* sp. (4.9%) etc. (Table 1). The secondary pollen types recovered from Suruk honey (SURH) of Kalimpong sub-division were *Rosa* sp. (24%) *Brassica* sp. (22.2%) and *Clematis* sp. (20.0%). The important minor pollen types were *Bauhinia* sp. (10.0%), *Solanum* sp. (7.5%) and *Holboelia latifolia* (7.0%).

From the present survey 60 pollen belonging to 32 families have been recorded (Table 2). Maximum number of pollen types (24) were recovered from Seed From honey of Kalimpong and minimum number (8) from Kurseong honey. The intermediate number of pollen types recovered were Dungra Busty – 10, Echhey Busty-14, Mongpu –19, Samsing –11, Suruk-12. Among the total 60 taxa which were found to be foraged by bees 52 taxa were entomophilous and rest 8 were amphiphilous (i.e. pollinated by both insect and air) in the present investigation.

The honey dew elements (HDE) represented by shreds of fungal hyphae and fungal spores like uredospores of *Puccinia*, conidia of *Fusarium* were recovered in very small amount from all the samples. HDE/P ratio 0.008-0.02 (Table 1) indicates that the honey dew elements were practically none (Louveaux *et al.*, 1978).

On the basis of Absolute Pollen Count (APC) of the seven samples the unifloral Echhey Busty Honey (EBH) was referable to Group II of I.C.B.B. (APC 23377/10 gm). The rest six samples were referable to Group I (Table 1).

The honey samples showed variation of colours from light yellow to amber due to different degree of pigmentation from visual observation point of view.

Based on the frequency distribution of pollen types on the honey samples four classes were recognised. "Very frequent" pollen types consisted of two only viz. *Rosa* sp. and *Aristolochia* sp. 'Frequent' pollen consisted of 9 types viz. *Brassica, Bidens, Tropaeolum Nicotiana, Calendula, Porana, Clematis, Dahlia* and *Bellis.* 'Infrequent' pollen consisted of 7 types like *Cardamine, Porana, Streptosolen, Mazus, Camellia, Bauhinia* and *Chrysanthemum.* 'Rare' pollen consisted of 42 types like *Michelia, Solanum, Buddleja, Dichroa, Luffa, Trifolium, Thunbergia, Coccinea, Euphorbia, Prunus, Oxalis, Primula, Holboelia, Lindenbergia,* etc.

DISCUSSION

Pollen analytical studies of honeys coupled with critical field observations help in providing data regarding the bee plants of an area and also the favourable period of honey production in commercial quantitites (Ramanujam & Kalpana, 1995). The present study highlights a number of plants serving as reliable sources of nectar for the honey bees of sub-Himalayan area during the period November 1996 to February 1997. *Rosa* sp., *Aristolochia* sp., *Brassica* sp., *Bidens pilosa, Cardamine hirsuta, Bellis perennis, Tropaeolum majus, Calendula officinalis, Clematis* sp. provide the major sources of nectar for the honey bees; *Bauhinia* sp., *Ageratum conyzoides, Erigeron karwinskianus, Buddleja asiatica, Trifolium repens, Primula* sp., Spirea spp., *Oenanthe thomsonii, Raphanus sativus, Dahlia imperialis, Camellia* sp., *Majus surculosus, Dichroa febrifuga, Porana* sp., etc. represent the other noteworthy sources of nectar.

The similarity index values in 5 pairs of samples (Table 3) were found to be lower than 0.5. This clearly indicates that they originated from the different areas and are attributable to different floristic composition

within the perimeter of the bees foraging range and diversity of pollen types originating from both wild and cultivated plants.

During the present survey some pollen types like *Gynocardia odorata, Bauhinia* sp. with their local names 'Gantay' and 'Varala' respectively were reported to render intoxicating properties to honeys from the local people. *Gynocardia* had been found in Suruk honey (SURH) as minor pollen type (0.31%) whereas *Bauhinia* had been found as important minor pollen type (10%) in the same honey. It needs further investigation in respect of fermentation of sugars in the nectar into some alcoholic compounds. Mention may be made also regarding the toxicity of honey due to toxic nectar and pollen sources. Florido-Lopez *et al.* (1995) reported the occurrence of allergy to natural honeys and camomile tea. In the present investigation *Croton* had been recovered as minor pollen type (1%) from Samsing honey. Toxic honeys collected during the blooming period of such poisonous plants should be avoided (Bera *et al.*, 1997).

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