

VIETNAM NATIONAL UNIVERSITY OF AGRICULTURE
RESEARCH CENTER FOR TROPICAL BEES AND BEEKEEPING



*Research on the origin of honey in
the market by pollen grain analysis
and atlas.*

Pham Hong Thai

INTRODUCTION

- ❑ Pollen grains (PGs) is the most important source of proteins for honeybees which them collected created pollen load (PLs).
- ❑ Various colors of PLs depending on the botanical taxa meaning it reflected relationship botanical taxa with PG collected by honeybees.
- ❑ Honey has a high nutrition value □ the demand for quantity and increasing.
- ❑ Product qualities have assurance on product's origin or not?
And Viet Nam often is victim of the transshipment honey of some yes again exporting to other countries, making reduction the brand quality Viet Nam' honey products on export markets.
- ❑ In Vietnam, the determination of original quality of honey based on analysis ingredients physico-chemical, sensory evaluation, not yet done in pollen analysis.
- Therefore, to responding above issue, the research with title: "Study on melissopalynology for evaluating original quality of honey in Ha Noi market."



MATERIALS AND METHODS

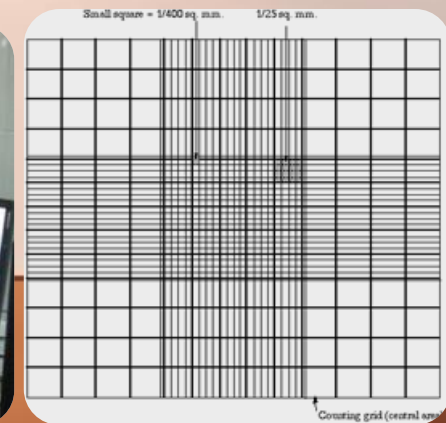
Materials

❑ **Pollen types on PLs** were surveyed and collected from 4 *Apis mellifera* colonies in *jujube* season at VNUA campus.



❑ **68 honey samples** collected from different ecological regions and sources such as: Dong Van, Moc Chau, Hung Yen, Bac Giang, honeys collected on Ha Noi market with different types, labels, date and manufactures, honeys collected from *Apis mellifera* honeybees in Hua campus, honey combs (honey without centrifuging from beekeepers), and honey collected from foreign country.

❑ **Tools for experiment in lab:** Slides, lamella, Neubauer hemocytometer, Zeiss microscope, distilled water, centrifuge machine, test-tubes, blotting- paper, alcohol 75% , dyes(methylene or glycerine jelly).



Overview methodology



Method S

1. Trapping PLs and classify color of PLs

Time

How to collect PLs

How to classify color of PLs

2. Collection honey in Ha Noi market and different ecological regions

3. Analysis and identify PGs

On PLs

Separated color PLs

Preparation slide

Identifications types of PGs

Isolation PGs

Preparation slide

Identifications and counting PGs

In honey

Data calculation

PGs size measurement

PGs counting

4. Calculation and report of results

Data report

Qualitative melissopalynology

Quantitative melissopalynology

Methodology (4 main contents)

1) *Trapping pollen and classify color of PLs from PLs mixture at VNUA campus*

☐ Time for collected PLs ?

One time in midmorning 9-11 hours, every 3-4 days.

☐ How to collect PLs ?

☐ How to classify color of PLs?

Using table code color.

2) *Collection honey in Ha Noi market and different ecological regions and sources.*



Fig 1. Trapping and collected PLs

Methodology

3) Analysis and identify PGs on PLs and in honey samples in lab.

☐ Analysis PGs on PLs.

- ☐ Separated color PLs from PLs mixture
- ☐ Preparation of microscope slide: diluted PLs to make a pollen solution and making specimen on slide.
- ☐ Observing and identify types of PGs under *Zeiss* microscope.

☐ Analysis PGs in honey samples

- ☐ Isolation of PGs from honey by centrifuging (Kerkvliet, 1995)
- ☐ Preparation of microscope slide: prepare solution sedimentation and making specimen on *Neubauer* hemocytometer. (BS111L Basic of using hemocytometer)
- ☐ Identification and counting of PGs by *Neubauer* hemocytometer through the microscope: (BS111L Basic of using hemocytometer)

Methodology

4) Calculation and report of results: relationship between pollen analysis and honey types.

☐ Data calculation

❖ *PGs size measurement* : $S = 0.0026 \times A$

❖ *PGs counting (Godini, 1979)*

$$N = \frac{n \times 1000}{A \times h \times 16 \times 25} = \frac{n \times 1000}{\frac{1}{400} \text{ mm}^2 \times 0.100 \text{ mm} \times 16 \times 25} = n \times 10\,000$$

☐ Data analyzing (Excel program) and report results

❖ *Qualitative melissopalynology*: PGs counted and identified.

❖ *Quantitative melissopalynology*: PGs counted

Report results *Qualitative melissopalynology*

- ❑ For research purpose or for honey less quality that honey were classified following the number of PGs in counting area.

Group I: Honey have number of PG in each sample from 0-50 PG in counting area in 10 gram honey, *Group II:* 51-100 PG, *Group III:* 101-150 PG , *Group IV:* 151-200 PG, *Group V:* more than 200 PG

- ❑ And then the following term are used:

+) Predominant pollen: if the percentage of the pollen type is more than 45%

+) Accompanying pollen or 2nd pollen : pollen type is 16-45%

+) Isolated pollen or importance minor pollen: pollen type is 3-15%

+) Minor pollen: pollen types is less than 3%

Report results *Quantitative melissopalynology*

Honey samples fall into one of the following groups:

- +) **Group I** : < 20.000 pollen per 10g honey poor in pollen
- +) **Group II** : 20.000- 100.000 pollen per 10g : most honey type obtained by centrifuging
- +) **Group III** : 100.000 – 500.000 pollen per 10g honeys from flowers rich in pollen
- +) **Group IV** : 500.000- 1.000.000 pollen per 10g honey extremely rich in pollen and some pressed honeys
- +) **Group V** : > 1.000.000 pollen per 10g: pressed honeys.

RESULTS

Determine pollen types on PLs

- 29 different pollen types of plant species (excluding unidentified) were identified of which 23 types had percentages higher than 1%, and only 1% of the total PLs were unidentified. Overall 5 of them are identified to family, 5 of them to genus and to 18 species level.
- There were many characteristics of PG was recorded (Sawyer,1988) such as: shape(round, triangular, polyad...), size, surface, aperture type, exine, section and other structure features .

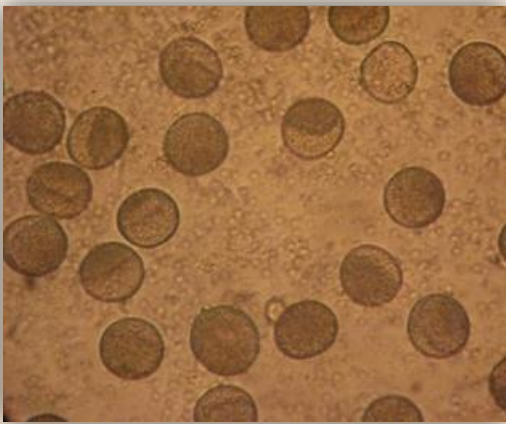


Fig 2. *Oryza sativa* (Rice) PG
(Mag. 40) Size: 36-39 μm - medium

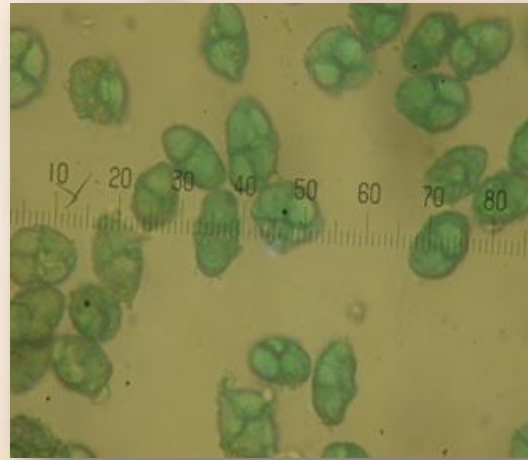


Fig 3: *Mimosa pigra* PG
(Sensitive plants) (Mag. 40)
Size: 26-30 μm - small

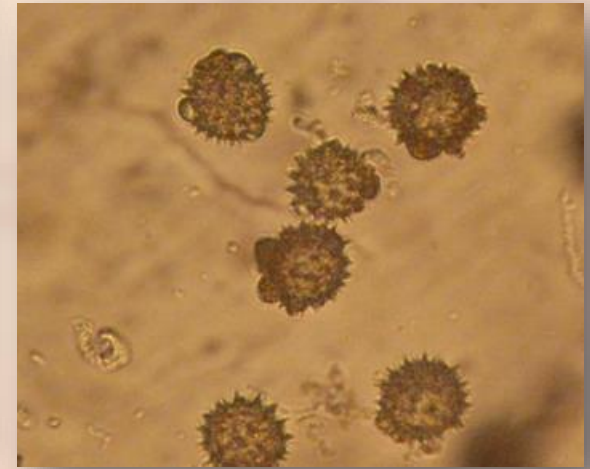


Fig 4: *Bidens pilosa* PG
(Mag.40) Size: 22-26 μm - small

Note: Abbreviate letter were name type of plants species and families PG

A	<i>Biden pilosa</i> (Càng cua)	I	<i>Styphnolobium japonicum</i> (hoa hòe)	Q	<i>Psidium guajava</i> (Ổi)	Y	Rosaceae (Họ hoa hồng)
B	<i>Eclipta alba</i> (Nhọ nồi)	J	Fabaceae (Họ đậu)	R	Myrtaceae (Họ sim)	Z	<i>Schefflera octophylla</i> (Ngũ gia bì)
C	<i>Brassica juncea</i> (chinese cabbage)	K	<i>Mimosapigra</i> (Trinh nữ đầm lầy)	S	<i>Nelumbo nucifera</i> (Lotus)	A1	<i>Ziziphus mauritiana</i> (Táo)
D	<i>Brassica rapa</i> (spinach mustard)	L	<i>Mimosapudica</i> (Trinh nữ)	T	<i>Nymphaea rubra</i> (hoa súng)	B1	Umbelliferae (Họ hoa tán)
E	<i>Acacia spp.</i> (Chi keo)	M	Lamiaceae (Họ hoa môi)	U	<i>Zea mays</i> (Ngô)	C1	Unknown
F	<i>Bursera spp.</i> Burseraceae (Họ trám)	N	<i>Michelia alba</i> (Ngọc lan)	V	<i>Rice Oryza sativa</i> (Lúa)		
G	<i>Phaseolis vulgaris</i> (đậu cô ve).	O	<i>Muntingia calabura</i> (Trứng cá)	W	<i>Ludwigia spp.</i> Onagraceae (Họ rau dĩa)		
H	<i>Copiapoa spp.</i> Cactaceae (Ho xương rồng)	P	<i>Eucalius spp.</i> Myrtaceae (Ho sim)	X	<i>Polygonum spp.</i> Polygonaceae (ho dăm dai)		

4.1.1 Treatment number types of pollen collected by each honeybee colonies.

Table 1: Number pollen types collected by *Apis mellifera* honeybee colonies in Hua campus in *jujube* season, 2011

No. of colonies	Number types of pollen collected by honeybees
M1	27
M2	21
M3	21
M4	25

- Table 1 showed that there were variance number of plants collected by *Apis mellifera* honeybees among colonies.
- But treatment between number of plant species collected among colonies showed that there were no significant differences in power plants provide pollen for honeybees.

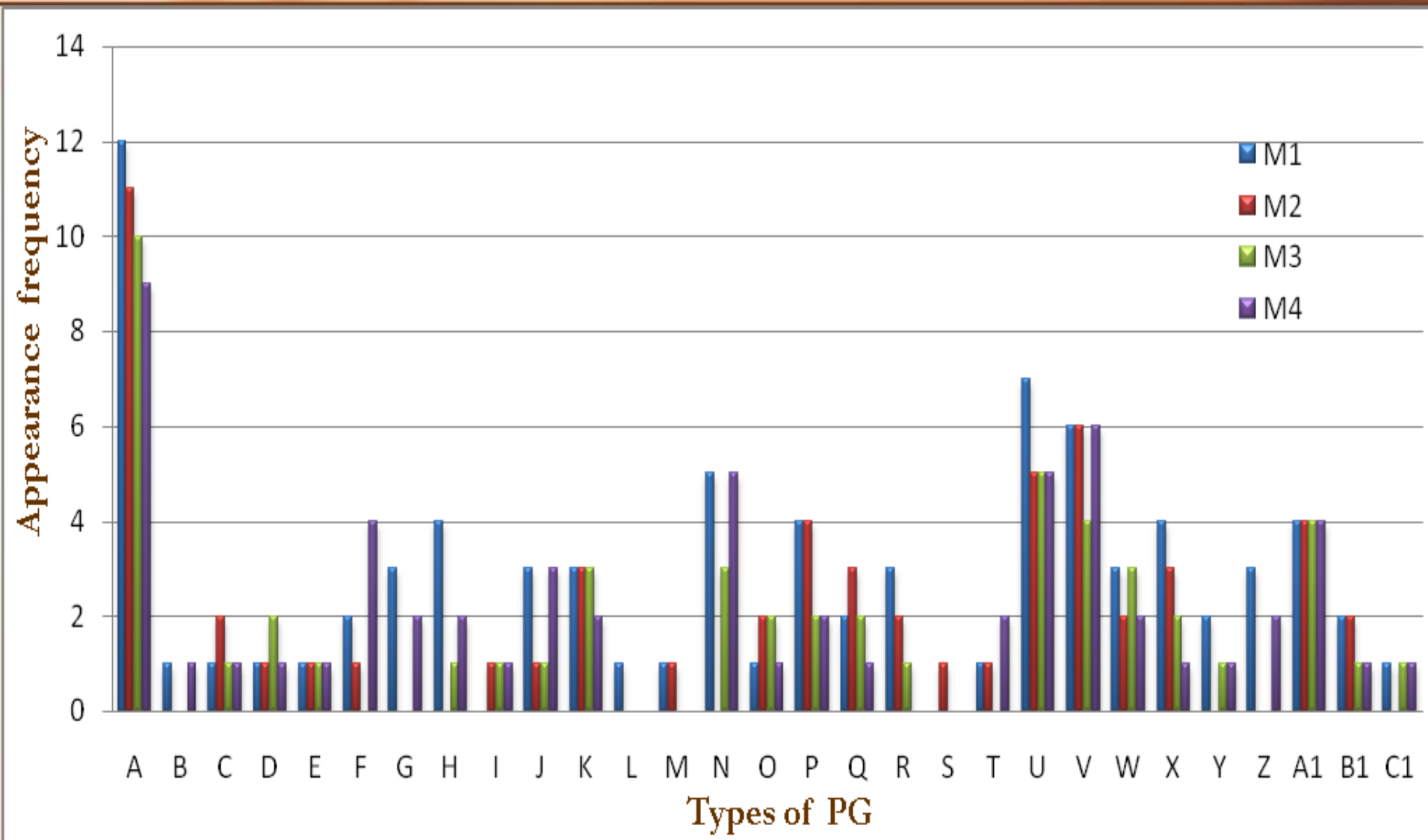


Fig 5: Appearance frequency pollen types on PLs of colonies during days studied in *jujube* season in VNUA campus

- ❑ *Bidden pilosa* were plants had the highest appearance frequency, next to *Zea mays* , *Rice Oryza sativa* , *Michelia alba* , *Ziziphus mauritiana* other species with small amount.
- ❑ And LSD 1%, $P = 0.002$, $F_{tn} = 20.17$, $F(0.05, 28, 65) = 1.64$ were treatment between number of plants collected by 4 colonies also showed that there were meaning differences in power plants provide pollen for honeybees

- ❑ Number kinds of PGs in a day collected by 4 colonies were not similar.
- ❑ Treatment between number of pollen types in a day studied which collected by 4 colonies showed that there were no significant differences.
- ❑ Results of Anova analysis pollen types in different days studied showed that there were significant differences between species PGs with LSD 1%, $P = 0.008$, $F_{tn} = 3.21$, $F(0.05, 28, 105) = 1.58$.
- ❑ Plants were as sources supply pollen for honeybees had change in the end in Sept., mostly *Corn* and *Rice*.
- ❑ Observed show that there were not much *Jujube* PG collected by honeybees. It means that in the *jujube* season, *jujube* were not a major source of pollen for honeybees.

Overview honey groups

68 honey samples belong to 7 main honey groups



Honey collected from honeybees at Hua campus (3 samples)

Elsho. honey group (13 samples)

Longan honey group (11 samples)

Litchi honey group (5 samples)

Bidden honey group (9 samples)

Honey group which collected on Ha Noi market (21 samples)

Honey group which collected from foreign country (4 samples)



Honey group had label "*Forest blossom honey*" (6 samples)

Honey group had label "*Royal jelly of honey*" (6 samples)

Honey group had label "*natural honey*" (6 samples)

Honey group had label "*special honey*" (3 samples)

4.2 Melissopalynology(M)(Pollen analysis in honey)

4.2.1 (M) from observed colonies at VNUA(3 samples)

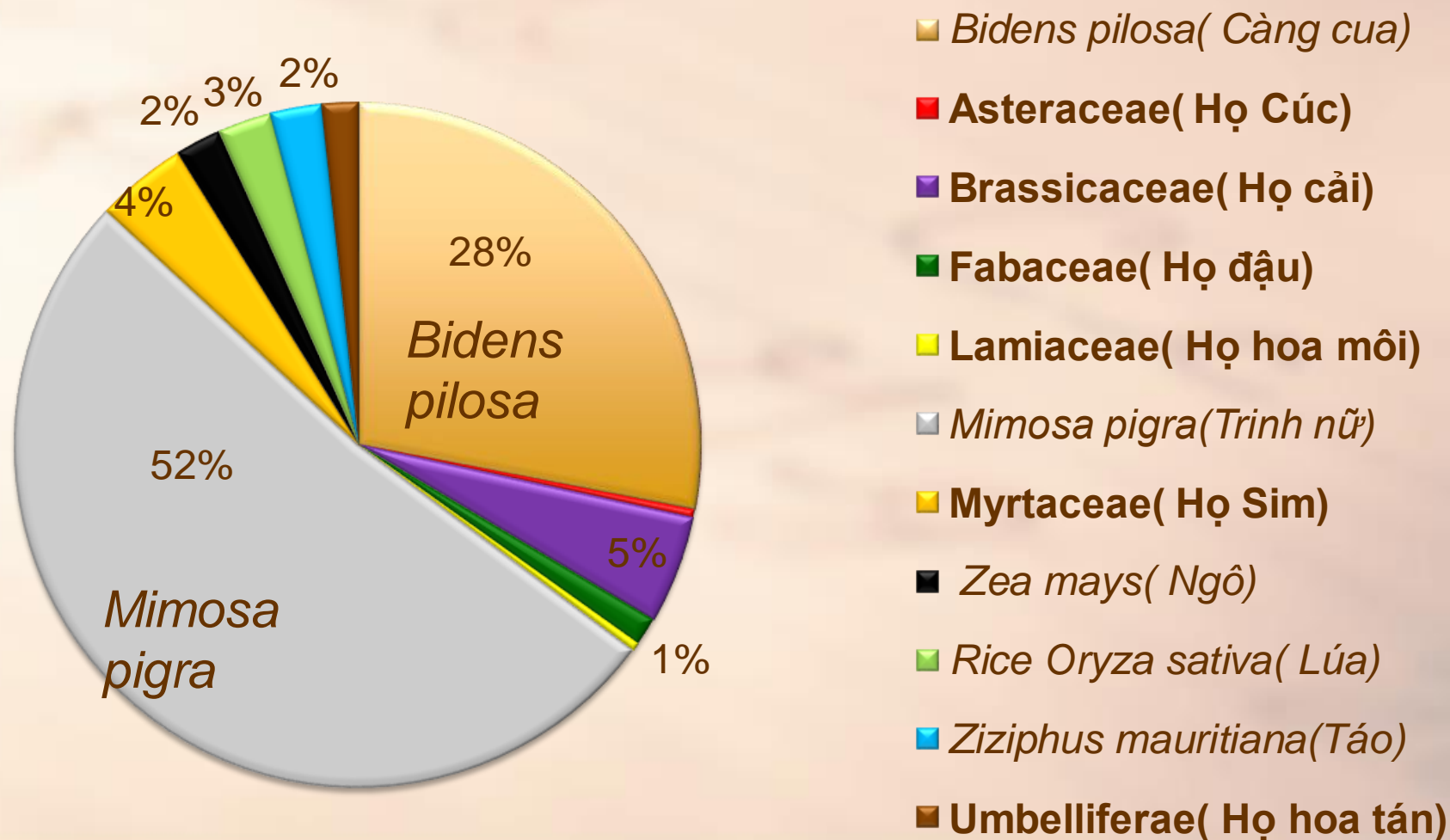


Fig 7: The ratio pollen types in counting area in honey from collected *Apis mellifera* honeybee colonies in Vnua campus

- ❑ Melissopalynology results showed that 11 plant species and their families were indentified in this honey group:
- ❑ And Anova analysis showed there were significant differences between these pollen types with LSD 1%, $P = 0.008$, $F_{tn} = 105, 5$, $F(0.05, 10, 203) = 1.8$
- ❑ Big pollen appearance frequency on testing solution were PGs collected the day before centrifuging and also had the highest number of PG in counting area .(*Mimosa spp.*)
- ❑ The number of *jujube* PG also came into view but with little amount. However, it as well showed out that *jujube* had been as source supply pollen for honeybees.

4.2.2 Melissopalynology of *Elsho. honey* group samples (13 samples)

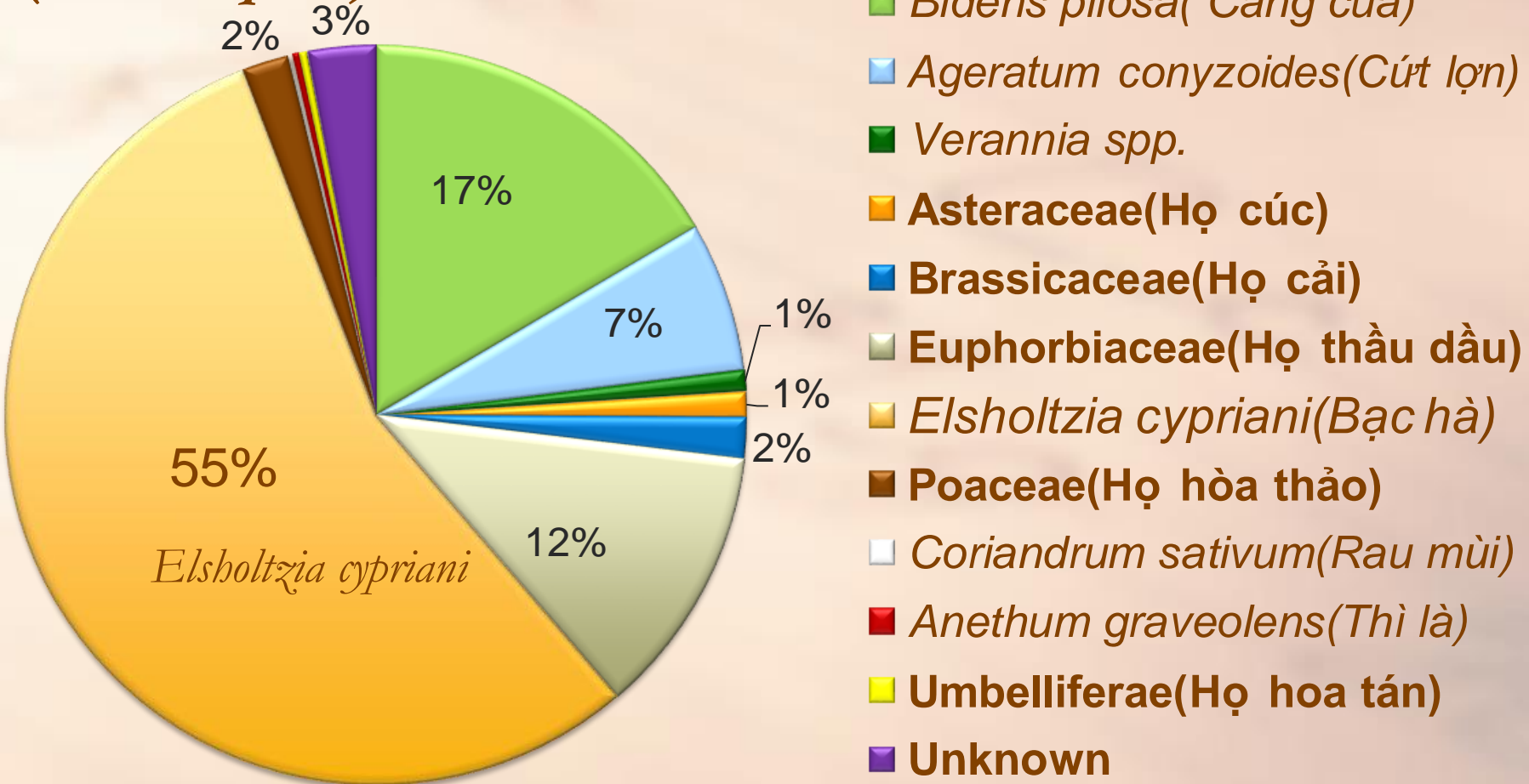


Fig 8: The ratio pollen types in counting area in *Elsho. honey* group collected in Ha Giang and on market.

- ❑ (M) results showed that 13 plant species and their families(including unknown) were indentified.
- ❑ *Elsho.* PG had the biggest quantity with 55%, next to *Bidden pilosa* 17%, ***Euphorbiaceae*** 12%, ***Poaceae*** 7%
- ❑ *Elsho.* honey purchased on the market had number of PG smaller than honey collected in Ha Giang province.
- ❑ Compared *Elsho.* honey : Qualitative melissopalynology:

Elsho. PG (Mag. 40)

Shape: Oval flattened

Size: 20- 33 μm - small

Surface: Indefinite

Aperture number: 6 furrows

Exine, section: Close, thin rod



4.2. 3 *Melissopalynology with Longan honey group samples (11 samples)*

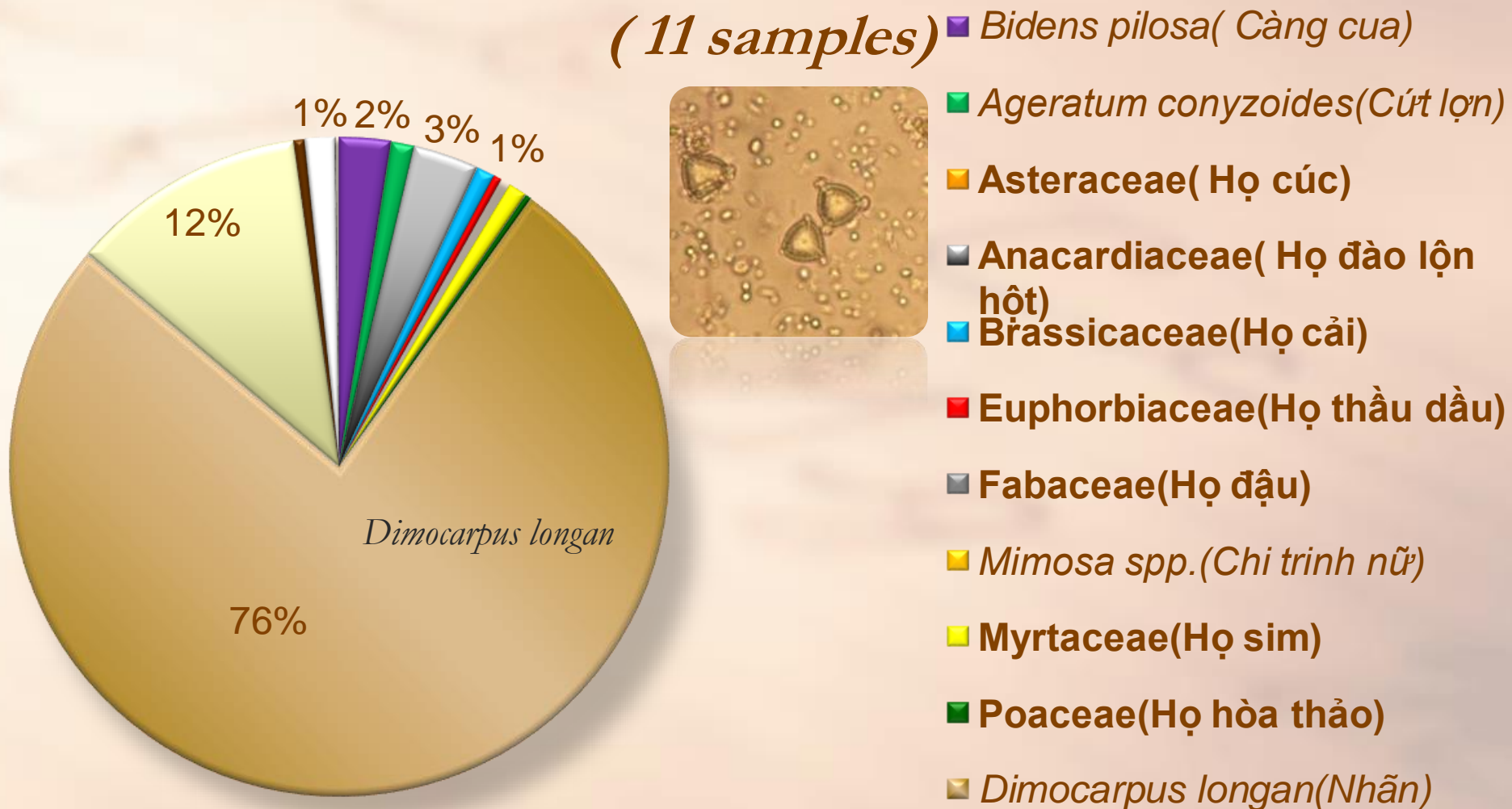


Fig 9: The ratio pollen types in counting area in *Longan* honey group.

- ❑ 16 plant species and their families (include unidentified) were indentified in this honey group.
- ❑ and *Longan* PG had the highest amount and appearance frequency in *longan* honey samples (11 samples).
- ❑ Qualitative melissopalynology: showed there were no significant differences between *Longan* honey samples collected from provinces but quantitative melissopalynology showed *longan* honey samples collected in Hung Yen had the highest number of PG in honey contrast with other regions: Hai Duong, Moc Chau, Hua university, Nghe An , Yen Bai
- ❑ There no significant differences between the time centrifuging in the same *longan* season (Qualitative melissopalynology) by contrast, the number of PGs found in honey (quantitative melissopalynology) was different.

4.2.4 *Melissopalynology with Litchi honey group samples.* (5 samples)

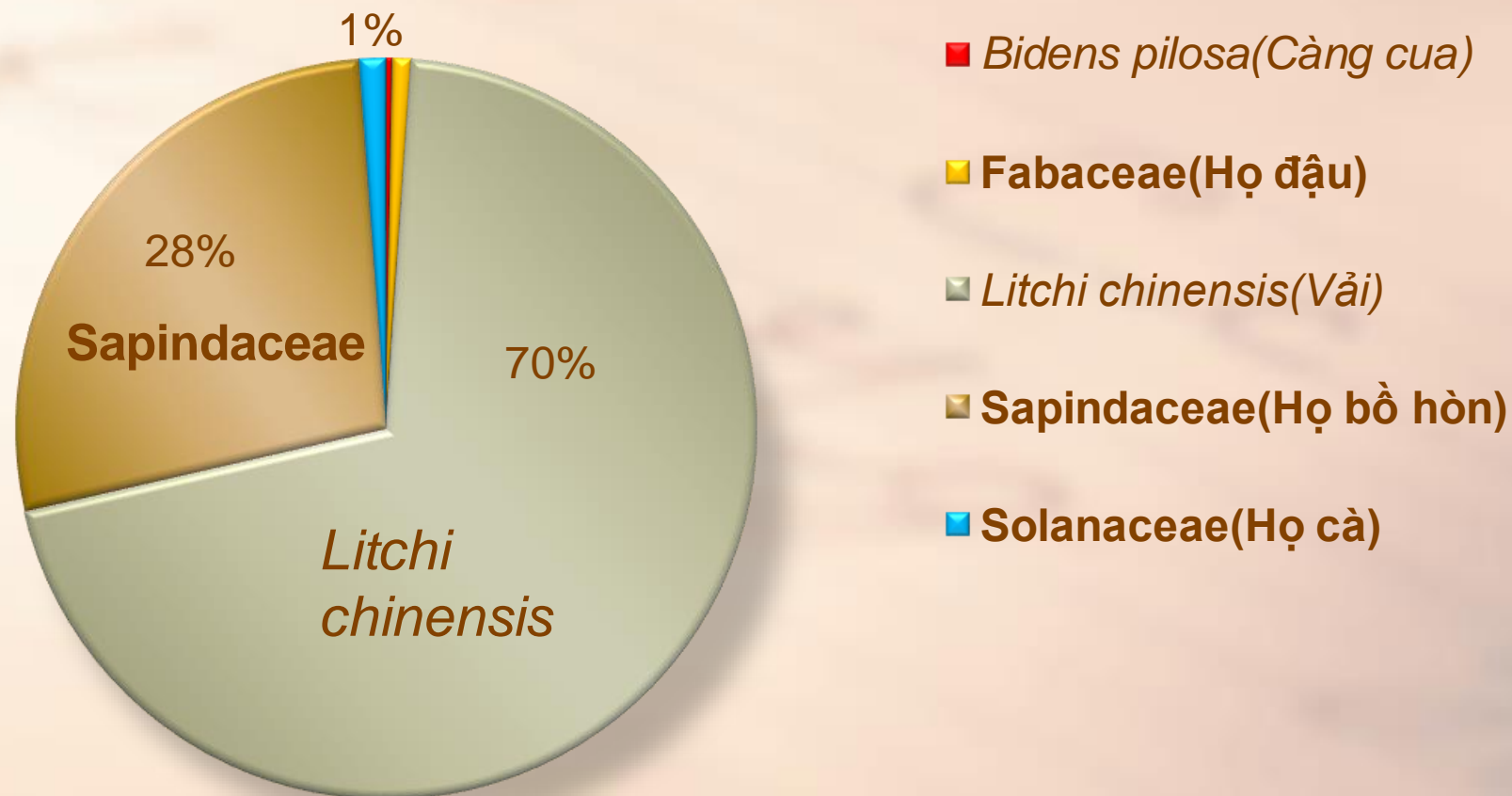


Fig 10: The ratio pollen types in counting area in *Litchi* honey in Bac Giang

- ❑ For 5 *Litchi* honey samples collected in Bac Giang that 2 plant species and 3 families were indentified in this group in that *litchi* PG had the highest amount occupied to 70%, next to *Sapidaceae* 28%.
- ❑ Anova analysis showed that there were meaning differences between types of PG in honey (LSD 1%, P= 0.0007, F_{tn}= 12.4, F (0.05, 4, 10) = 3.4.
- ❑ It also showed that *litchi* were as the main source supply pollen for honeybees with the highest average number of *litchi* pollen in honey.

4.2.5. *Melissopalynology with Bidden honey group samples (9 samples)*

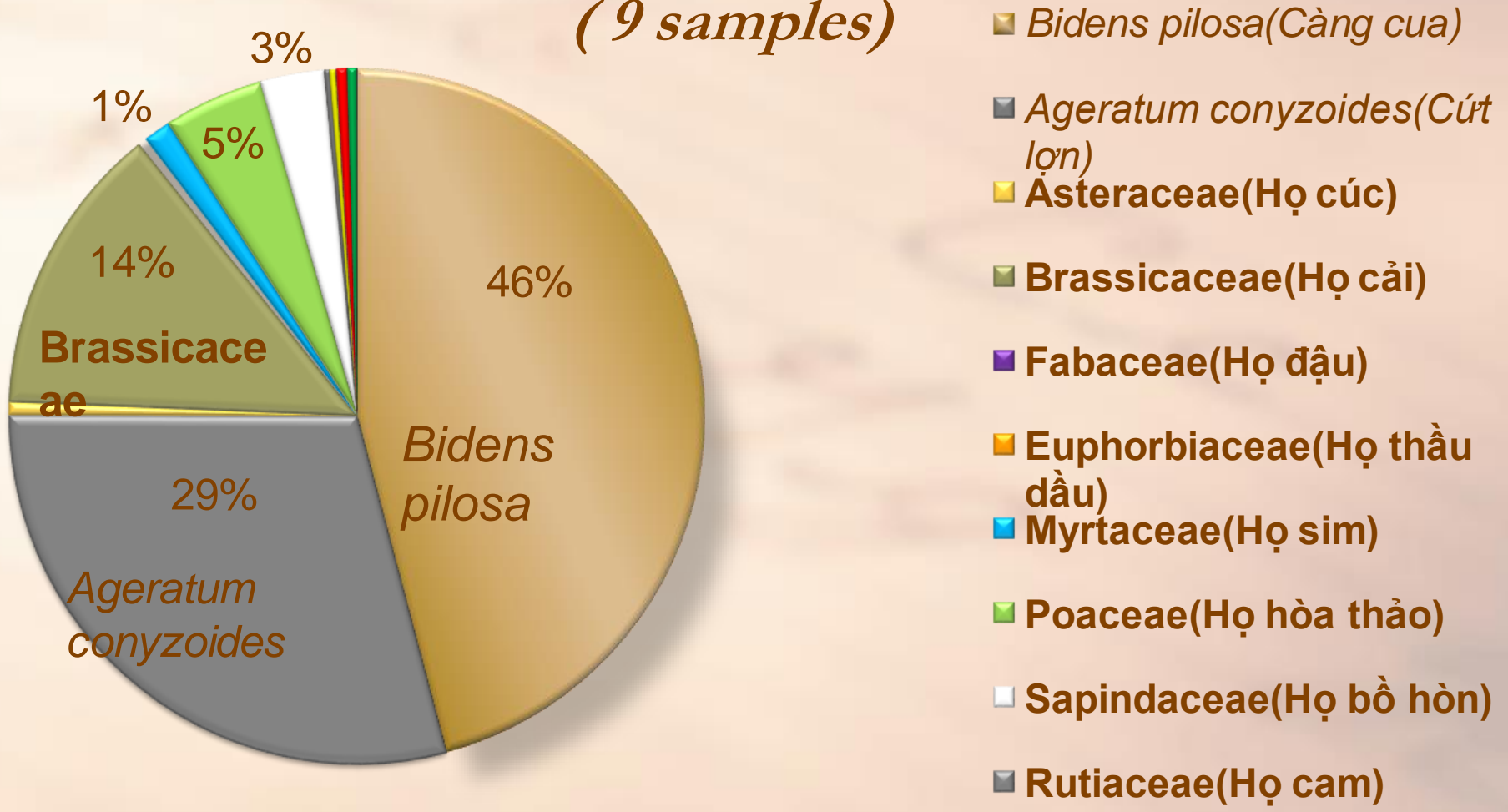


Fig 11: The ratio pollen types in counting area in *Bidden* honey in Son La

- ❑ 13 plant species and their families were indentified in this honey group.
- ❑ And *Bidden* PG get the highest proportion 46%, followed by *Ageratum conyzoides* 29%, ***Brassicaceae***14% and ***Poaceae*** 5% but Anova analysis showed that there were no meaning differences between types of PG plant species in honey.
- ❑ The average number of *Bidden* PG in this honey group not much
- ❑ the number of pollen were not huge such that these honey may be didn't has exactly origin from Son La or it were mixed with other types of honey.

4.2.6 *Melissopalynology of collected honey group in Ha Noi market (21 samples)*

- ☐ There were the biggest diversity types of PG compared with other above honey groups.
- ☐ *Longan* were plants had the highest proportion.
- ☐ The numbers of PGs in this honey groups little than above honey groups. Whatever pollen is the source of food for honeybees so that pollen were exist in honey even more or less
- ☐ To evaluate these honey by melissopalynology methods, beside base on information record on label, we have based on special types of pollen grains were found in honey.

Honey group had label "Forest blossom honey" (6 samples)

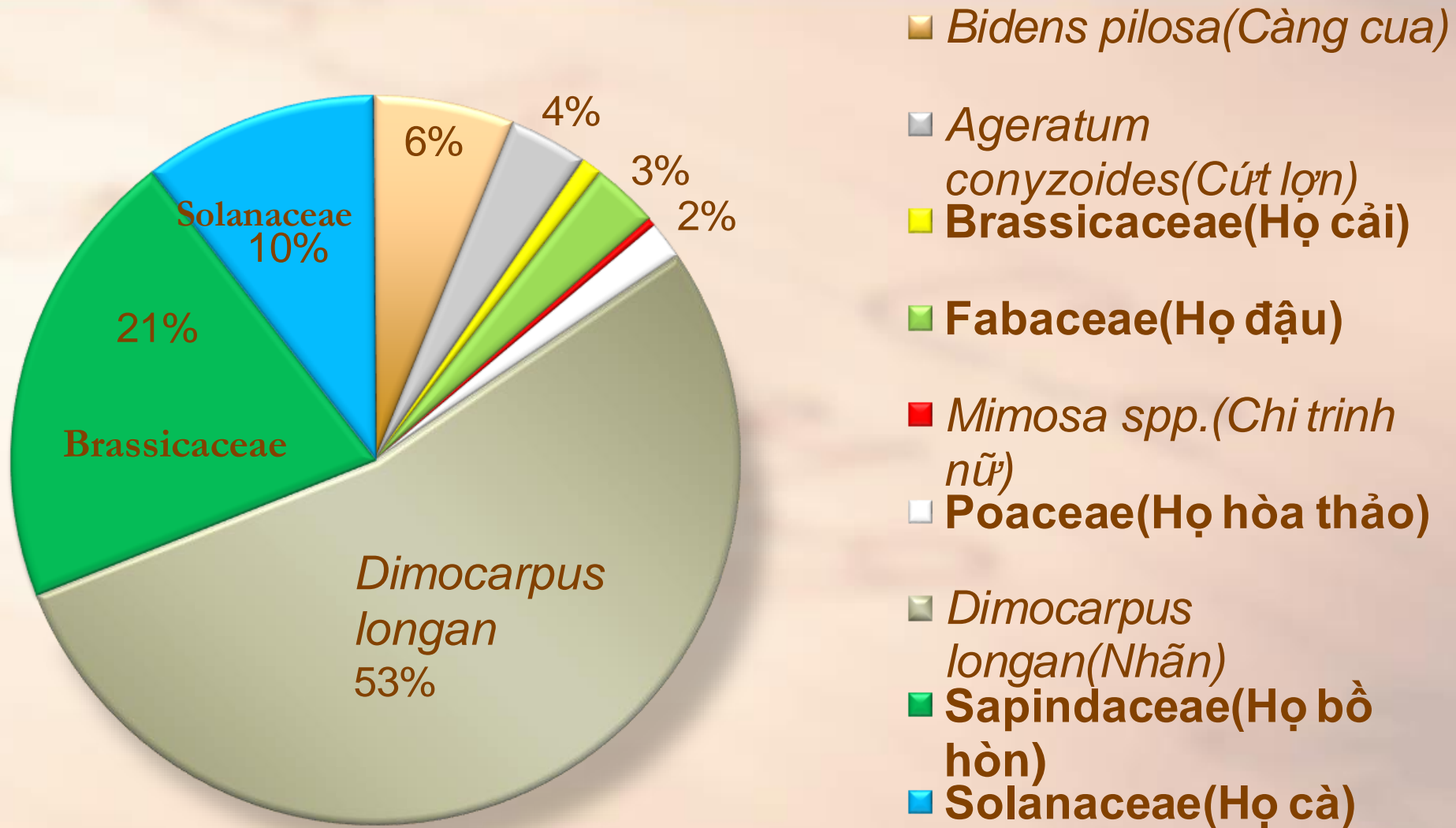
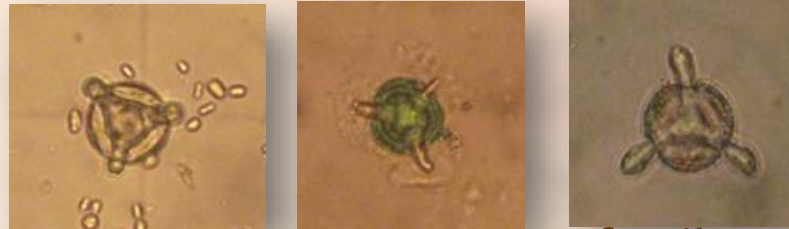


Fig 12: The ratio pollen types in counting area in forest honey collected in market

☐ 10 species and families (including unknown) were found in counting area in this group.

☐ And special pollen of genus *Corylus spp.* (family ***Betulaceae***) were found in 2/6 honey samples



☐ 2/6 remain samples were found PG of ***Pineceae*** family.

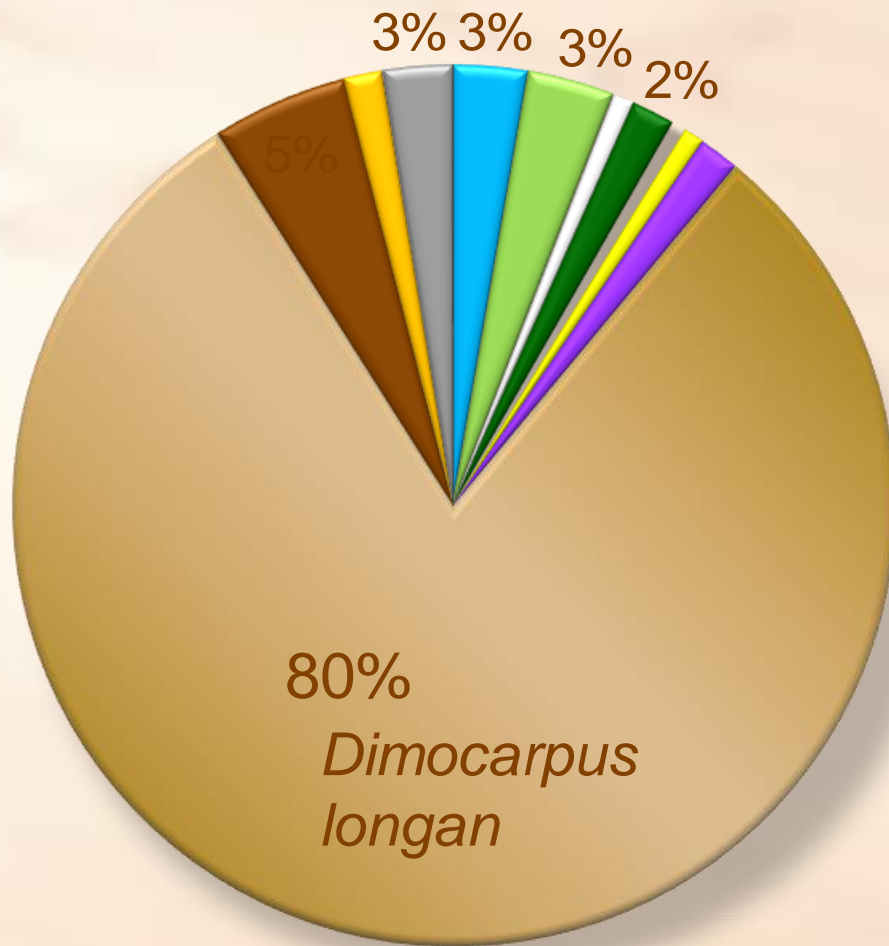


☐ “U Minh forest honey” which botata by AN Ti Co., Ltd, the kind of ***Rhizophoraceae*** (**ho duoc**) PG were found in honey.



☐ Just only 1/6 did not find any specific pollen .

Honey group had label "Royal jelly of honey" (6 samples)

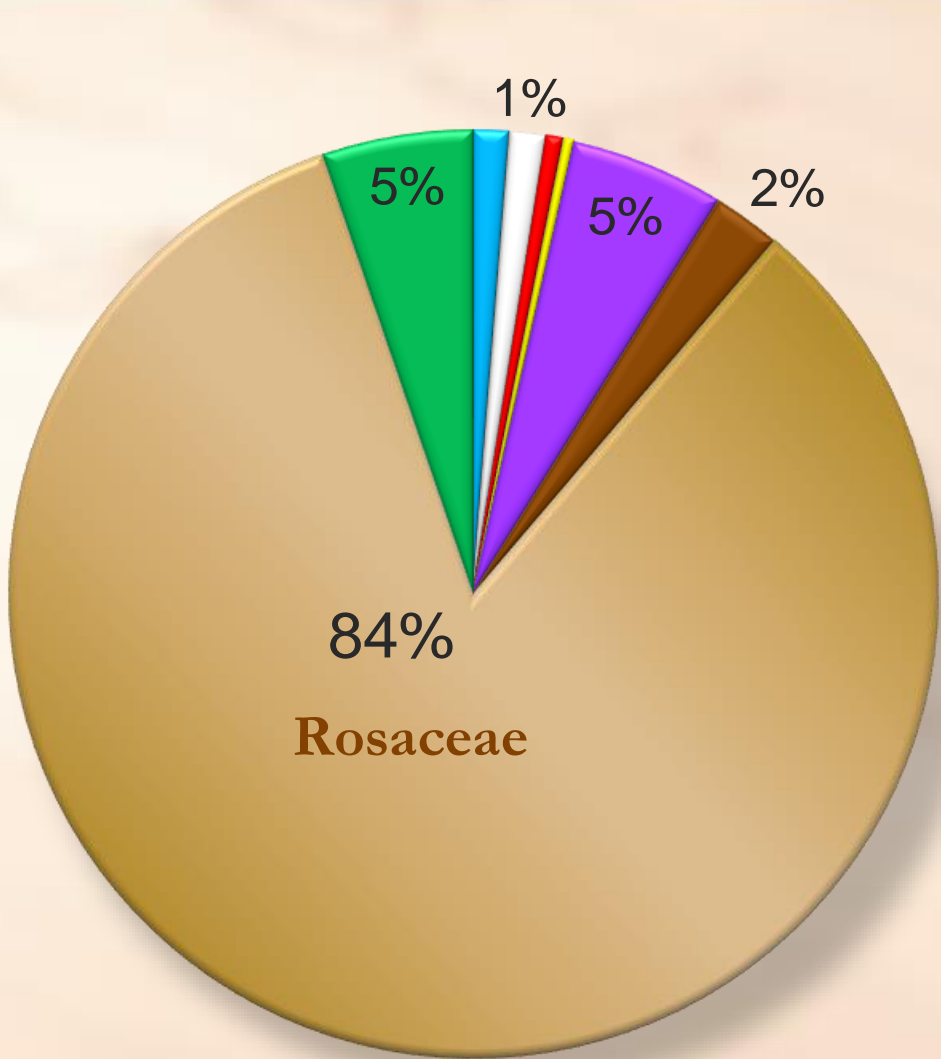


- *Bidens pilosa* (Càng cua)
- *Ageratum conyzoides* (Cút lợn)
- *Asteraceae* (Họ cúc)
- *Anacardiaceae* (họ đu đủ)
- *Fabaceae* (Họ đậu)
- *Mimosa spp.* (chi trinh nữ)
- *Myrtaceae* (Họ sim)
- *Poaceae* (Họ hòa thảo)
- *Dimocarpus longan* (Nhãn)

Fig 12: The ratio pollen types in counting area in *Royal jelly* honey group collected in market

- ❑ This honey group determined by this method is to determine them closely with what kind of unifloral, floral or plant types.
- ❑ 3/6 royal jelly honey samples showed that them closer *longan* honey.
- ❑ 3/6 remain still found PGs of some families such as ***Asteraceae***, ***Euphorbiaceae***, ***Myrtaceae***, ***Poaceae*** but the number of PG found in these sample were little and each types of occupy with small proportion.
- ❑ These honeys may be filter before bottles or even may be sugar fed honey. So to evaluate these samples, it needs to combine with other methods.

Honey group had label "natural honey " (6 samples)



- Bidens pilosa(Càng cua)
- Ageratum conyzoides(Cút lợn)
- Anacardiaceae(Họ đào lộn hột)
- Brassicaceae(Họ cải)
- Poaceae(Họ hòa thảo)
- Sapindaceae(Họ bồ hòn)
- Solanaceae(Họ cà)
- Rosaceae(Họ hoa hồng)
- Umbelliferae(Họ hoa tán)

Fig 12: The ratio pollen types in counting area in honey purchased samples on market

- ❑ The common species and families were found in honey such as: *Asteraceae*, *Myrtaceae*, *Brassicaceae*, *Fabaceae*, *Sapidaceae*, *Sonalaceae*, *Euphorbiaceae*, *Poaceae*, *Umbelliferae*, *Roseceae*
- ❑ Most of them had small number of PG in counting area in honey.
- ❑ And none special PG were discovered except “Pure natural blossom honey - Le fruits” samples bottled by “Trà Nóc 1-Cần Thơ industry park”.
- ❑ The biggest number of pollen types in counting area in honey belong to *Rosaceae* (occupied to 93%)



Honey group had label "special honey" (3 samples)

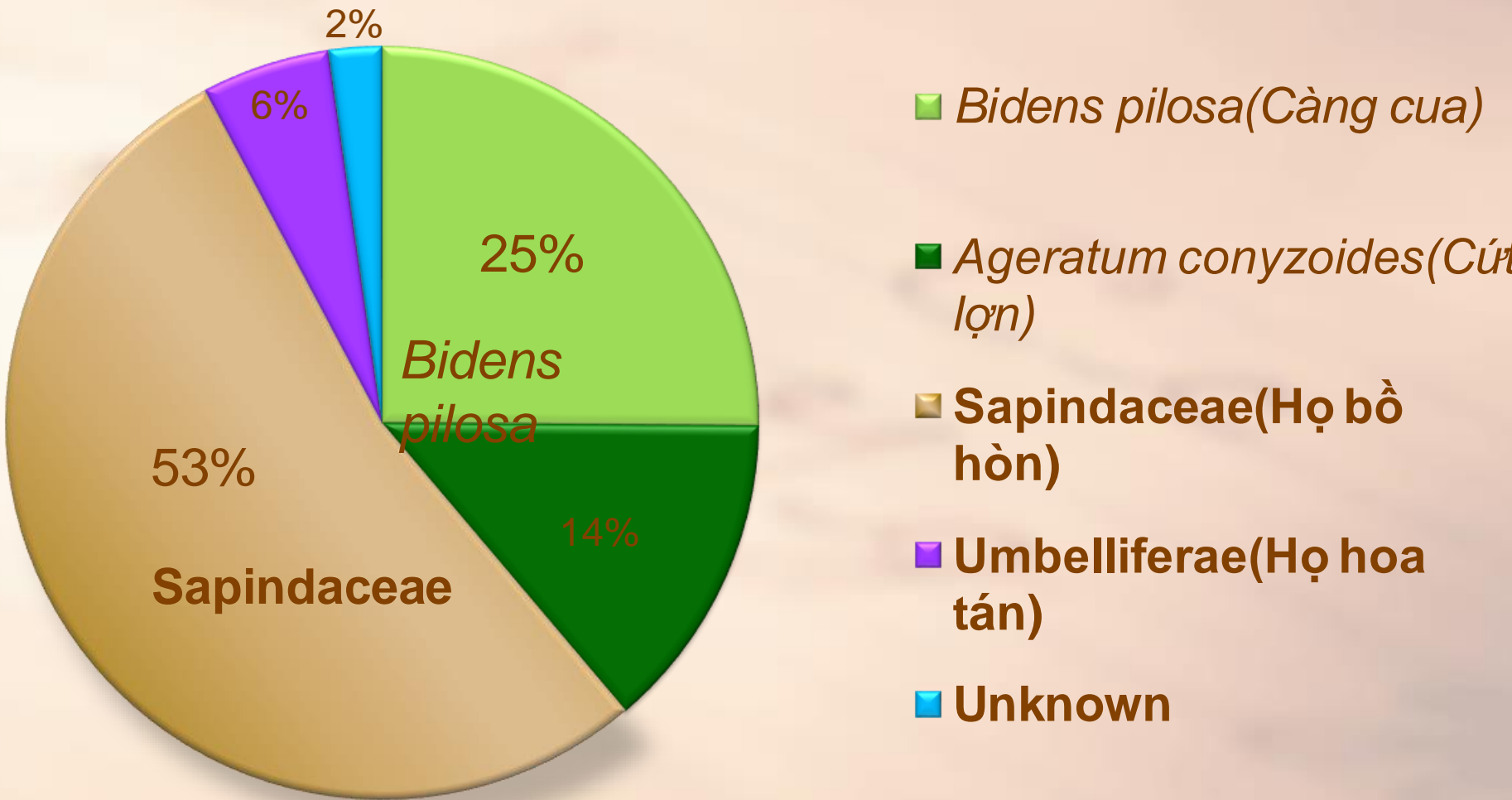


Fig 12: The ratio pollen type in counting area in honey purchased samples on market

- ❑ Many pollen types were found in these samples (7-15 types) but the number of PG in counting area just had only 3-5 types and amount of PG in counting area also little .
- ❑ Special PG belong to genus *Corylus spp.*(family ***Betulaceae***) as well found in 2/3 samples.

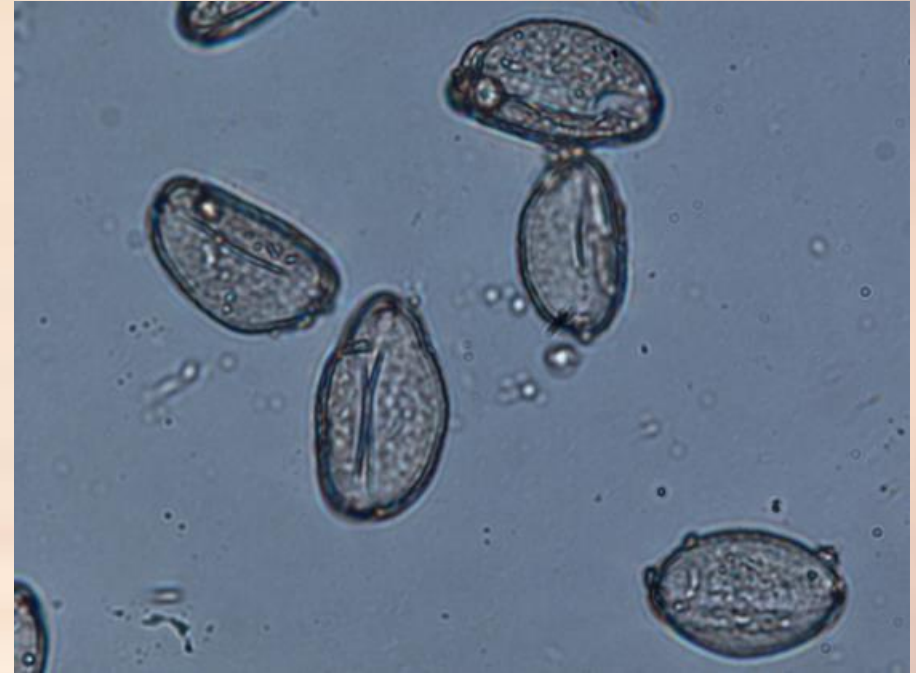
Atlas of pollen grains

Onion



Scientific name: *Allium cepa*

- Common name: Onion, bulb onion
- Order: Alliaceae
- Family: Alliaceae
- Genus: *Allium*



- The pollen grain of onion (mag.100)
- Shape: Semi-circular or board shape
 - Size: 31 μm -medium
 - Surface: Indefinite
 - Aperture type: Furrows only
 - Exine, section : Medium, no rods

Coriander



- Scientific name: *Coriandrum sativum*
- Common name: Coriander
- Order: Apiales
- Family: Umbelliferae
- Genus: Coriandrum

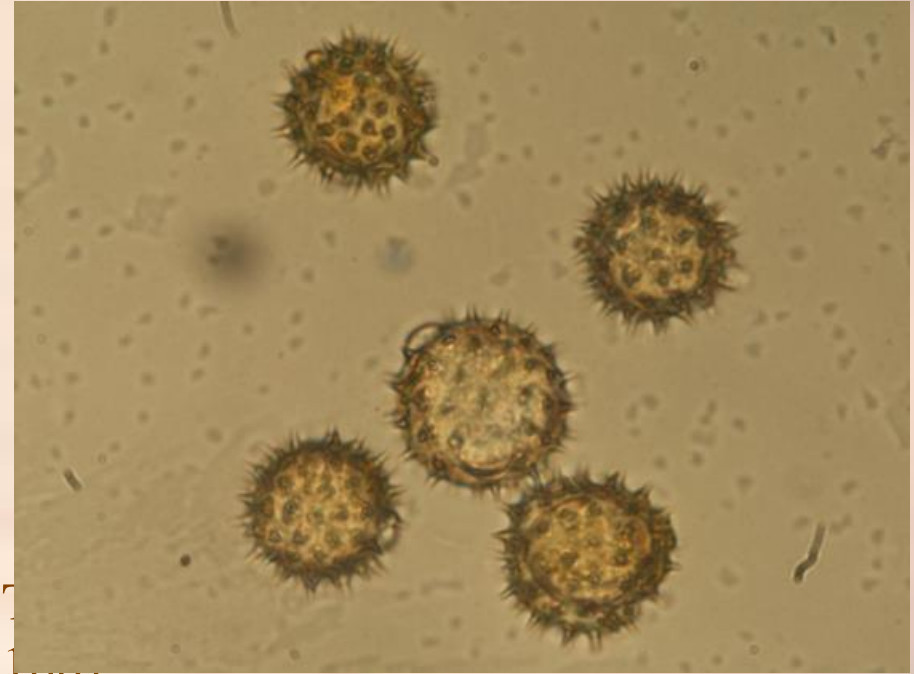


- Shape: Long
- Size: 31 μm -medium
- Surface: Indefinite
- Aperture type: Irregular furrows may occur
- Exine, section : Thin
- Other structure features : Edges thickened

Spanish Needle

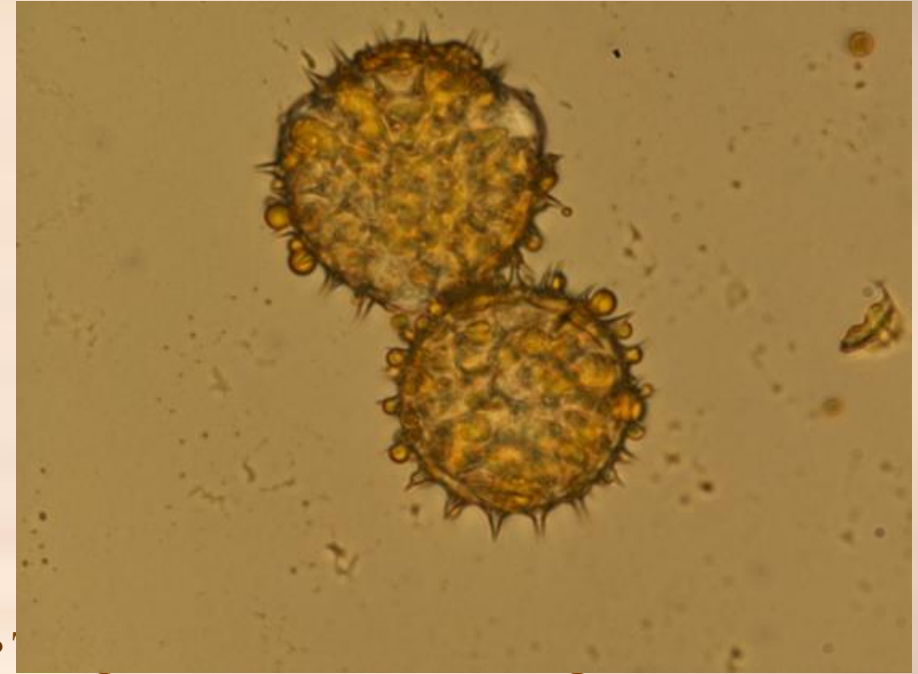


- Scientific name: *Bidens pilosa* L.
- Common name: Spanish Needle
- Order: Asterales
- Family: Asteraceae
- Genus : Bidens



- Shape: Round or irregularly round
- Size: 23 μm - small
- Surface: Dots due to spines
- Aperture type: No furrows
- Exine, section: Long, thin spines

Sunflower

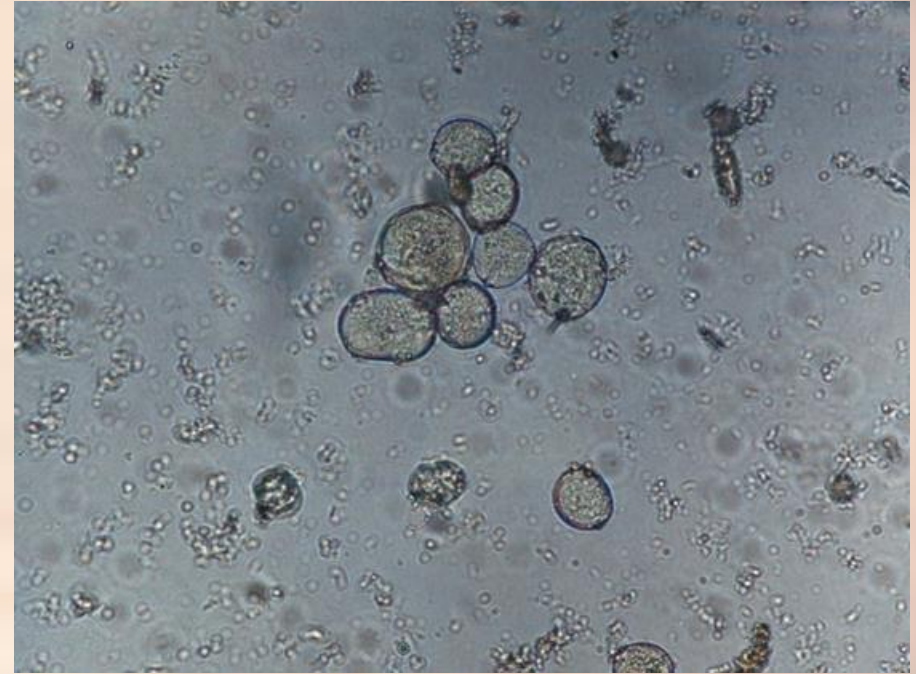


Scientific name: *Helianthus annuus*

- Common name: Sunflower
- Order: Asterales
- Family: Asteraceae
- Genus: Helianthus

- Shape: Round
- Size: 38 μm - medium
- Surface: Dots due to spines
- Aperture type: Irregular furrows may occur
- Exine, section : Long, thin spines
- Other structure features : oil layer on surface

Longan



Scientific name: *Dimocarpus longan*

- Common name: Longan
- Order: Sapindales
- Family: Sapindaceae
- Genus: Dimorcarpus

The pollen grain of Longan (mag. 40)

- Shape: Irregular round or round triangular
- Size: 23 μm - small
- Surface: indefinited or smooth
- Aperture type: united
- Exine, section : thin
- Other structure features : cap or streak

Litchi



- Scientific name: *Litchi chinensis*
- Common name: Litchi
- Order: Sapindales
- Family: Sapindaceae
- Genus: Litchi

The pollen grain of Litchi(mag. 40)

- Shape: Rounded triangular
- Size: 26 μm - small
- Surface: Smooth or indefinite
- Aperture type: Unitted
- Exine, section :Thin
- Other structure features : cap or streak

Thanks for your attention!

