Sociobiology
An international journal on social insects

RESEARCH ARTICLE - BEES

Pollen Content in Honey of *Apis mellifera* Linnaeus (Hymenoptera, Apidae) in an Atlantic Forest Fragment in the Municipality of Piracicaba, São Paulo State, Brazil

DFD Araujo[1], ACCC Moreti[2], TA Silveira[1], LC Marchini[1], IP Otsuk[2]

1 - Universidade de São Paulo, ESALQ/USP, Piracicaba-São Paulo, Brazil.

2 - Secretaria de Agricultura e Abastecimento, Nova Odessa, São Paulo, Brazil.

Article History
Edited by
Celso F. Martins, UFPB, Brazil
Received 13 may 2013
Initial acceptance 26 June 2013
Final acceptance 18 October 2013

Keywords
Melissopalynology; Nectar; Foraging; Apiculture; Food source.

Corresponding author:
Diogo Feliciano Dias Araujo
Departo. de Entomologia e Acarologia
Universidade de São Paulo
Campus "Luiz de Queiroz"
Avenida Pádua Dias, 11, São Dimas, 13418-900, Piracicaba, São Paulo - SP, Brazil
E-Mail: diogofdaraujo@gmail.com

Abstract

The productive and reproductive characteristics of *Apis mellifera* L. bees are directly affected by climatic conditions and food availability in the region where the bees are reared or kept. Therefore, food storage (honey and pollen), oviposition and population growth of these bees are subject to seasonal variations. These variations lead the bees to constantly search for food, making exploratory trips, called "foraging". This study investigated the botanical origin of nectar sources collected by *A. mellifera* bees for six consecutive months, from October/2011 to March/2012 in six bee colonies. The study was carried out in the experimental apiary of the Entomology and Acarology Department of the College of Agriculture "Luiz de Queiroz", from the University of São Paulo, in the municipality of Piracicaba, São Paulo State. The study site has a predominant vegetation of Semideciduous Forest (Atlantic Forest). In each sampling month, we analyzed the pollen types in the honey samples. We used the acetolysis method to prepare the samples for melissopalynology. We carried out the quantitative analysis by successive count of 900 sample grains. The samples were grouped in terms of botanical species, families and/or pollen types. The results show that bees used several plants from the region as a nectar source. However, the Arecaceae, Fabaceae/Mimosoideae and Myrtaceae families were predominant throughout the sampling period. The occurrence of these plant species was significant and essential for the maintenance of the bee colonies.

Introduction

The population growth of a bee colony depends entirely on the quality and quantity of nectar and pollen sources collected by workers, since honey and pollen, a carbohydrate and a protein source, respectively, are essential for the nutrition of larvae and adults of *Apis mellifera* (L.) (Zerbo et al., 2001). The composition and quality of food sources as well as other factors that affect the colony growth may vary according to the region and year season (Funari et al., 2003; Marchini et al., 2006).

To obtain an adequate food storage and high population growth rate, colonies need to build an optimal food inventory. The climatic conditions and food availability in the region directly affects the productive and reproductive characteristics of bee colonies. Therefore, food storage (honey and pollen), oviposition and occupation of combs are subject to seasonal variations (Modro et al., 2011).

The biological characterization of the honey plays an important role in ecological and commercial aspects, contributing to the process for quality control of the honey and even to the standardization to honey products used in food and pharmaceutical industries (Lins et al., 2005; Neves et al., 2009).

Melissopalynology is the science that deals with the morphological characteristics of pollen grains and spores, as well as their dispersion and use (Barth, 1989). It is a very important tool to identify plant species visited by bees. This science branch contributes to research by providing information on the food source location and allows to identify the range that bees travel to collect food in the year seasons. This study investigated the pollen in honey produced by *A. mellifera* bees during six consecutive months, between October 2011 and March 2012.

Material and Methods

Study site

We carried out the experiment at the experimental apiary of the Laboratory of Beneficial Insects from the Entomology and Acarology Department of the College of Agriculture "Luiz de Queiroz", at the University of São Paulo. The research facility is located in the municipality of Piracicaba, São Paulo State at 22º 42' 02" S / 47º 37' 35.18" O, at 539 meters above sea level. The site has predominant vegetation of Semideciduous Forest (Atlantic Forest).

Honey bee colonies

We installed six colonies of Africanized bees (*A. mellifera*) in Langstroth hives. Initially, we set up a nest for oviposition and pollen and nectar storage for colony maintenance. During the sampling period, we added supers to collect honey. Ten days before each collection, the supers were added to the colonies for a complete clean and reconstruction of alveolus and later honey storage.

Sampling

The samples were collected on a monthly basis from October 2011 to March 2012. Afterwards, we added the supers to the nests, each with ten plots, and carried out inspections every15 days. In case there was honey deposited into the supers, we removed it, bagged it individually in sterilized plastic bags, centrifuged it, stored about 200g in sterile plastic flasks and placed in acclimatized chambers of BOD (Biologic Oxygen Demand) at constant temperature of 20 °C, with the respective identification of each colony.

Melissopalynological analyses

The melissopalynological analyses of the samples were carried out according to the standard methodology using acetolysis (Erdtman, 1952). The identification of the pollen types was based mainly on the reference collection of microscopy blades from the Pollen Database of the Laboratory of Beneficial Insects of the Entomology and Acarology Department of College of Agriculture "Luiz de Queiroz". We also used specialized catalogs of pollen morphology of several floral species (Barth, 1989; Roubik & Moreno, 1991; Carreira et al., 1996; Colinvaux et al., 1999; Moreti et al., 2002; Carreira & Barth, 2003; SANTOS, 2006). The term used for identification of pollen types is not related to the International Code for Botanical Nomenclature; however, it gives an approximate identification of the samples with an existing taxonomic group (Joosten & Klerk, 2002). The scientific nomenclature used follows the norms proposed by APG II (Angiosperm Phylogeny Group II), according to Souza and Lorenzi (2008), and the nomenclature is in accordance to Tropicos.Org (2011). We identified and counted about 900 pollen grains per sample and each pollen grain was photomicrographed using a Zeiss photomicroscope.

After counting the pollen grains, we grouped them according to the following international criteria: Predominant Pollen (PP) – more than 45% of total pollen grains counted; Accessory Pollen (AP) – from 16 to 45%; Isolate Pollen (IP) – up to 15%, subdivided into: Important Isolate Pollen (IIP): 3 to 15% and Occasional Isolate Pollen (OIP): less than 3% (Louveaux et al., 1978).

Data analysis

The experimental design was completely randomized with six replications (represented by the colonies) and data were repeated to study the families and pollen types of the plants visited by the bees during the six months of study.

After classification, the data were analyzed through the MIXED procedure of the SAS (Statistical Analysis System), SAS Institute (2001) to determine structure of matrix for variance and covariance at 5% probability. The data were converted to square root of (X+1) to obtain homocedasticidity in the analysis of variance.

Results and Discussion

We identified 26 pollen types in the honey samples (Table 1) belonging to 15 botanical families and the families Arecaceae, Fabaceae and Myrtaceae (Figure 1) were the main food sources identified. Comparing our findings to results obtained by Modro (2011) and Silveira et al. (2012), carried out in the same region to identify pollen types collected by *A. mellifera* bees for one year, in our study, we observed a smaller number of pollen types collected by this bee species. The authors identified between 60 and 80 pollen types; however, despite the smaller number of pollen identified, the melissopalynological analysis in study was carried out in two seasons, showing an even greater diversity of botanical species visited by the bees during the experiment. The variation in foraging of the botanical sources used by the bees, regardless the source collected (honey or pollen), can vary according to several factors such as flowering, climate and competition with other bee species (Gonzalez et al., 1995; Villanueva & Roubik 2004; Webby, 2004; Keller et al., 2005).

The pollen type from the Arecaceae family was found in the months of November and December of 2011 and January of 2012. However, its occurrence was AP only in November and December of 2011, remained as AP and IP in the other months. This is surprising because species of this family normally have greater occurrence during palynological studies on pollen types collected by the bees. The Arecaceae family shows a high pollen production rate due to the pollination syndrome occurring in most species (Barfod et al., 2011). However, bees collect

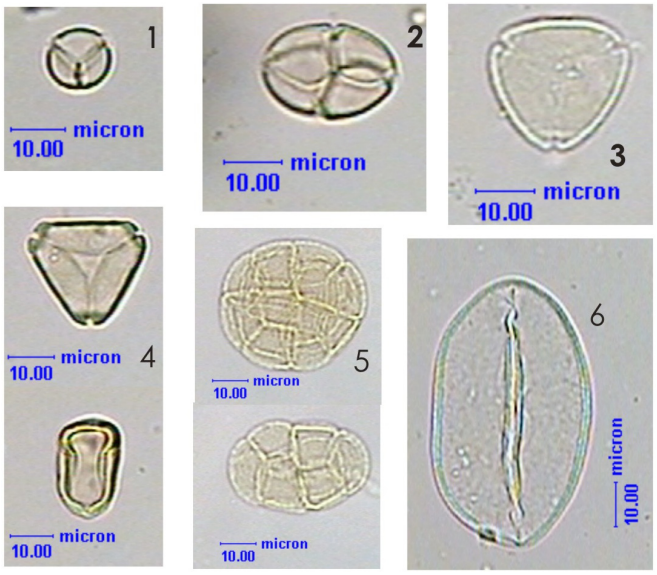

Figure 1 – Representation of the main pollen types found in the honey samples, collected from October/2011 to March/2012 from the Atlantic Forest fragment in the municipality of Piracicaba, São Paulo State, Brazil. (1) *Mimosa scabrella* Type; (2) *Mimosa caesalpinifolia*; (3) *Myrcia* Type; (4) *Eucalyptus* sp.; (5); *Anadenanthera* sp.; (6) Arecaceae Type.

nectar from Arecaceae because, in addition to great pollen production, its flowers are used in nectar production (Küchmeister et al., 1997; Mantovani & Morellato, 2000; Venturieri, 2008; Kidyoo & McKey, 2012).

The Arecaceae species are also important pollen sources for bees. This can contaminate honey and mask the significance of their relative high occurrence in honey samples. Similarly, many *Mimosa* species provide pollen grains that are identified in the melissopalynological analysis. Despite its importance for honey characterization, *Mimosa* is not the main nectar source collected for honey production. This fact may explain the occurrence of pollen types of the Arecaceae family during the palynological studies in honey samples. The Arecaceae family is an important food source for bees in the study area.

The pollen types of the Fabaceae family, such as that of *Anadenanthera* sp., showed greater occurrence in October/2011, and did not show significance in the other months. The *Mimosa scabrella* type was significant only in October/2011 and December/2011. In the same family, *Mimosa caesalpinifolia* was PP and AP in the months of February and March/2012, respectively, presenting itself as an important food source for all colonies, given that in February, this species was PP in 83.33% of the colonies, according to the classification of Louveaux et al. (1978). The same condition was found in March.

In the Myrtaceae family, we observed two pollen types, *Eucalyptus* sp. and *Myrcia*. The *Myrcia* type presented itself as an important food source for the bees, because this family showed significant occurrence (PP and PA) from October/2011 to January/2012, and can be considered one of the main species used for colony maintenance. *Eucalyptus* sp. showed occurrence after January/2012 and also considered an important food source for bees during the period.

Other studies in the region showed that the Fabaceae/Mimosoideae and Myrtaceae families are the main food sources for *A. mellifera* bees, showing the importance of this species for the colonies, both as nectar and pollen sources during certain periods of the year (Modro et al., 2011).

We identified species that were relevant as food sources based on the pollen types found, such as plants with potential use in management programs of apiculture in pasture. Despite the occurrence of other pollen types, bees showed intensive collecting activities of these plant species.

In a study on geographic location of food sources used by bees, the pollen considered as isolated occurrence can also serve as an important indicative to monitor the site, even showing low relevance in terms of quantity of nectar collected.

Conclusion

Although poorly used as a nectar source, species from the Arecaceae family are important food sources for bees during some seasons in the year.

Species from the families Arecaceae, Fabaceae/Mimosoideae and Myrtaceae are the most indicated for the implementation or improvement of apiculture pastures in the region.

The family Myrtaceae, represented by the pollen type *Myrcia*, which encompasses the genera *Psidium, Eugenia, Myrcia* and *Myrciaria*), show great importance as food source during the productive period of bees in the region.

Acknowledgments

The authors wish to thank CNPq for granting a scholarship to the first author.

References

Barfod, A.S., Hagen, M.,Borchsenius, F. (2011). Twenty-five years of progress in understanding pollination mechanisms in palms (Arecaceae). Ann. Bot., 108(8): 1503-1516. DOI: 10.1093/aob/mcr192.

Barth, O.M. (1989). *O pólen no mel brasileiro*. Rio de Janeiro: Gráfica Luxor.

Carreira, L.M.M.& Barth, O.M. (2003). *Atlas de pólen da vegetação de Canga da Serra dos Carajás, Pará*. Belém: Museu Paraense Emilio Goeldi.

Carreira, L.M.M., Silva, M.F. da, Lopes, J.R.C., Nascimento, L.A.S. (1996). *Catalogo de pólen das leguminosas da Amazônia Brasileira*. Belém: Museu Paraense Emilio Goeldi.

Colinvaux, P., Oliveira, P.E. de, Patiño, J.E.M. (1999). *Amazon pollen manual and atlas*. Amsterdam: Harwood Academic.

Erdtman, G. (1952) *Pollen morphology and plant taxonomy: Angiosperms*. Stockholm: Almqvist & Wiksell.

Funari, S.R.C., Rocha, H.C., Sforcin, J.M., Curi, P.R., Funari, A.R.M., Orsi, R. O. (2003) Efeitos da coleta de pólen no desenvolvimento de colônias e na composição bromatológica de pupas de *Apis mellifera* L. Arch. Latinoam. Prod. Anim., 11 (2): 80-86.

Gonzalez, A., Rowe, C.L., Weeks, P.J., Whittle, D., Gilbert, F.S. Barnard, C.J. (1995). Flower choice by honey bees (*Apis mellifera* L.): sexphase of flowers and preferences among nectar and pollen foragers. Oecologia, 101: 258-264.

Joosten, H. & Klerk, P. (2002) What´s in name?some thoughts on pollen classification, identification, and nomenclature in quaternary palynology. Rev. Palaeobot. Palynol., 122: 29-45.

Keller, I., Peter, F.,Imdorf, A. (2005) Pollen nutrition and colony development in honey bees: part I. Bee World, 86 (1): 3-10.

Kidyoo, A.M. & McKey, D. (2012) Flowering phenology mimicry on the rattan *Calamuscastaneus* (Arecaceae) in southern Tailand. Botany, 90: 856-865. DOI:10.1139/B2012-058

Küchmeister, H.,Silberbauer-Gottsberger, I.,Gottsberger, G. (1997) Flowering, pollination, nectar standing crop, and nectaries of *Euterpe precatoria*(Arecaceae), an Amazonian rain forest palm. Pl. Syst. Evol., 206: 71-97.

Lins, A.C.S., Silva, T.M.S., Camara, C.A., Dórea, M.C. Santos, F.A.R., Silva, R.A. (2005) Estudo químico, análise palinológica e atividade antiradicalar do pólen apícola (*Apis mellifera*). Soc. Bras. Quim. http://sec.sbq.org.br/cd29ra/resumos/T1270-1.pdf

Louveaux, J., Maurizio, A., Vorwohl, G. (1978). Methods of Melissopalynology. Bee World, 59: 139-157.

Mantovani, A. & Morellato, L.P.C. (2000) *Fenologia da floração, frutificação, mudança foliar e aspectos da biologia floral do palmiteiro*. In: Reis, M.S. & Reis, A. (Eds.), Euterpe edulis Martius – (Palmiteiro): biologia, conservação e manejo (pp. 283-301). Itajaí: Herbário Barbosa Rodrigues.

Marchini, L.C., Reis, V.D.A. & Moreti, A.C.C.C. (2006) Composição físico-química de amostras de pólen coletado por abelhas africanizadas *Apis mellífera* (Hymenoptera: Apidae) em Piracicaba, estado de São Paulo. Cienc. Rural, 36: 949-953.

Modro, A.F.H. (2011) *Influência do pólen sobre o desenvolvimento de colônias de abelhas africanizadas (Apis mellifera L.)*. Tese (Doutorado) - Escola Superior de Agricultura "Luíz de Queiroz", Universidade de São Paulo. Piracicaba, 2011, 98p.

Modro, A.F.H., Message, D., Luz, C.F.P. da., Meira Neto, J.A.A. (2011) Flora de importância polinífera para *Apis mellifera* (L.) na região de Viçosa, MG. Rev. Árvore, 35: 1145-1153.

Moreti, A.C.C.C., Marchini, L.C., Rodrigues, R.R.; Souza, V.C. (2002) *Atlas do pólen de plantas apícolas*. Rio de Janeiro: Papel Virtual.

Neves, L.C., Alencar, S.M., Carpes, S.T. (2009) Determinação da atividade antioxidante e do teor de compostos fenólicos e flavonoides totais em amostras de pólen apícola de *Apis mellifera.* Braz. J. Food Technol., 8: 107-110.

Roubik, D.W., Moreno, J.E.P. (1991) Pollen and spore of barro Colorado Island. St. Louis: Missouri Botanical Garden, (Monographs in Systematics Botany, 36). 268 p.

Santos, F.A.R.(Ed.) (2006). *Apium plantae*. Recife: Imsear.

Sas Institute. (2001) *SAS/STAT software: changes and enhancements through release 8.02.* Cary, 1167 p.

Silveira,T.A.,Correia-Oliveira,M.E.,Moretti,C.C.C.A.,Otsuk, I.P., Marchini, L.M. (2012) Botanical origin of protein sources used by honeybees (*Apis mellifera* L.) in Atlantic Forest. Sociobiology, 59(4): 1-10.

Souza, V.C., Lorenzi, H. (2008) *Botânica sistemática: guia ilustrado para identificação das famílias fanerógamas nativas e exóticas do Brasil, Baseado em APG II.* (2. ed.) Nova Odessa: Intituto Plantarum.

Tropicos.Org. (2011) Missouri Botanical Garden. <http://www.tropicos.org>.

Venturieri, G. (2008) *Floral biology and management of stingless bees to pollinate assai palm (Euterpeoleracea Mart., Arecaceae) in eastern amazon*. In: Alvarez, C.A.B; Landeiro, M. Pollinators management in Brazil. Brasília: Ministério do MeioAmbiente, p. 10-13.

Villanueva, G.R. & Roubik, D.W. (2004) Why are African honey bees and not European bees invasive? Pollen diet diversity in community experiments. Apidologie, 35: 481–491.

Webby, R. (2004) Floral origin and seasonal variation of bee-collected pollens from individual colonies in New Zealand. J. Apic. Res., 43: 83-92.

Zerbo, A.C.,Moraes, R.L.M.S. & Brochetto-Braga, M.R. (2001) Protein requirements in larvae and adults of *Scaptotrigona postica* (Hymenoptera: Apidia, Meliponinae) midgutproteolytic activity and pollen digestion. Comp. Biochem. Physiol., 129, p. 139 -147.

Table 1. Mean rate (%) of pollen types in honey samples of *Apis mellifera* collected from fragments of the Atlantic Forest in the municipality of Piracicaba, São Paulo State, Brazil, from October 2011 to March 2012. Means followed by different uppercase letters in columns differ in the Tukey-Kramer test (P<0.05). 1 Means between parenthesis are converted to root (X+1). #statistics referring to converted data. Predominant Pollen (*PP*); Accessory Pollen (*AP*); Isolate Pollen (*IP*), subdivided into: Important Isolate Pollen (*IIP*) and Occasional Isolate Pollen (*OIP*).

Botany Family/ Pollen type	Sampling months					
	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
Alismataceae/ Alismataceae Type			0,73±2,22 *OIP* (1.25±0.27)BC			
Amarantaceae/ *Alternanthera ficoidea*		4,17±1,82 *IIP* (2,11±0,22)BCD		0,17±1,82 *OIP* (1,07±0,22)C		
Amarantaceae/ *Amarantus* sp.				0,40±1,99 *OIP* (1,16±0,24)C		
Anacardiaceae/ Anacardiaceae Type		0,20±1,99 *OIP* (1.08±0.24)CD	0,20±1,99 *OIP* (1.08±0.24)C			
Araceae/ Araceae Type			1.00±1,82 *OIP* (1.38±0.22)BC	16.83±1.82 *AP* (3.67±0.22)B		8.83±1.82 *IIP* (2.99±0.22)C
Arecaceae/ Arecaceae Type	1.17±1.82 *OIP* (1.44±0.22)CD	37.83±1.82 *AP* (6.21±0.22)A	34.33±1.82 *AP* (5.71±0.22)A	21.67±1.82 *AP* (4.47±0.22)B	13.83±1.82 *IIP* (3.78±0.22)BC	12.00±1.82 *IIP* (3.49±0.22)B
Bombacaceae/ *Pachira aquatica*			0.33±1.82 *OIP* (1.14±0.22)C	0.17±1.82 *OIP* (1.07±0.22)C		
Compositae/ *Mikania laevigata*					0.17±1.82 *OIP* (1.07±0.22)E	
Compositae/ *Parthenium* sp.			0.17±1.82 *OIP* (1.07±0.22)C			
Cruciferae/ *Raphanus* sp.	0.33±1.82 *OIP* (1.12±0.22)D					2.67±1.82 *IIP* (1.58±0.22)D
Fabaceae/ *Caesalpinia pelthophoroides*		0.67±1.82 *OIP* (1.23±0.22)CD	0.17±1.82 *OIP* (1.07±0.22)C	0.17±1.82 *OIP* (1.07±0.22)C		
Fabaceae / *Caesalpinia* sp.	4.50±1.82 *IIP* (2.29±0.22)CD	2.50±1.82 *OIP* (1.71±0.22)CD	1.83±1.82 *OIP* (1.59±0.22)BC	0.17±1.82 *OIP* (1.07±0.22)C		
Fabaceae/ *Cassia* sp.	1.83±1.82 *OIP* (1.62±0.22)CD			0,17±1,82 *OIP* (1,07±0,22)C		
Fabaceae/ *Centrosema* sp.	3.17±1.82 *IIP* (1.96±0.22)CD	3.50±1.82 *IIP* (2.10±0.22)BCD	2.67±1.82 *OIP* (1.89±0.22)BC	0.50±1.82 *OIP* (1.21±0.22)C		
Fabaceae/ *Anadenanthera* sp.	17.50±1.82 *AP* (3.90±0.22)B	4.50±1.82 *IIP* (2.31±0.22)BCD	4.17±1.82 *IIP* (2.23±0.22)BC	3.67±1.82 *IIP* (2.05±0.22)C		0.17±1.82 *OIP* (1.07±0.22)D
Fabaceae / *Leucaena* sp.	3.83±1.82 *IIP* (1.99±0.22)CD	5.67±1.82 *IIP* (2.53±0.22)BC	2.00±1.82 *IIP* (1.70±0.22)BC	1.50±1.82 *OIP* (1.57±0.22)C		0.50±1.82 *OIP* (1.21±0.22)D
Fabaceae / *Mimosa caesalpinifolia*				1.33±1.82 *OIP* (1.44±0.22)C	55.00±1.82 *PP* (7.47±0.22)A	28.50±1.82 *AP* (5.37±0.22)A
Fabaceae / *Mimosa scabrella* Type	5.00±1.82 *IIP* (1.96±0.22)CD		10.33±1.82 *IIP* (2.65±0.22)B	2.50±1.82 *OIP* (1.72±0.22)C	6.83±1.82 *IIP* (2.76±0.22)CD	1.67±1.82 *OIP* (1.53±0.22)D
Fabaceae/ *Macroptilium* sp.				0.33±1.82 *OIP* (1.14±0.22)C		
Lamiaceae/ *Hyptis* sp.						0.67±1.82 *OIP* (1.26±0.22)D
Malvaceae/ *Dombeya* sp.		0.40±1.99 *OIP* (1.15±0.24)CD				
Moraceae/ *Morus* sp.	0.17±1.82 *OIP* (1.07±0.22)D	0.17±1.82 *OIP* (1.07±0.22)D	0.17±1.82 *OIP* (1.07±0.22)C			
Myrtaceae/ *Myrcia* Type	54.17±1.82 *PP* (7.07±0.22)A	27.50±1.82 *AP* (5.31±0.22)A	34.67±1.82 *AP* (5.93±0.22)A	36.33±1.82 *AP* (6.03±0.22)A	2.00±1.82 *OIP* (1.68±0.22)DE	1.33±1.82 *OIP* (1.44±0.22)D
Myrtaceae / *Eucalyptus sp.*	7.83±1.82 *IIP* (2.80±0.22)BC	9.17±1.82 *IIP* (3.15±0.22)B	6.83±1.82 *IIP* (2.72±0.22)B	13.50±1.82 *IIP* (3.78±0.22)B	21.83±1.82 *AP* (4.77±0.22)B	42.67±1.82 *AP* (6.58±0.22)A
Rubiaceae/ Rubiaceae Type		0.50±2.22 *OIP* (1.21±0.27)CD				
Rutaceae/ *Citrus* sp.	0.17±1.82 *OIP* (1.08±0.22)D	0.17±1.82 *OIP* (1.07±0.22)D	0.50±1.82 *OIP* (1.19±0.22)C	0.33±1.82 *OIP* (1.14±0.22)C		