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RESEARCH ARTICLE

BEE STRENGTH, SEASONAL VARIATION AND THEIR IMPACT ON WAX AND MECHANICAL IMPURITIES OF PROPOLIS

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ABSTRACT

Propolis is a resinous substance collected by bees from plants around the have. It is masticated by the bees, salivary enzymes and beeswax added, then applied to the combs and walls of the hive, thereby insulating and reinforcing the hives Propolis impurities were more, when less propolis was collected by the bees during spring (60.53 and 61.50%) and Summer (41.06 and 42.26%) as compared to those of Autumn season (34.00 and 35.00 %) from respective places Srinagar and Coimbatore. Impurities in propolis sample from low bee population (5100-7300 bees/colony) were more (66.60 and 68.03%) as compared to the samples from stronger colonies (38.66 and 39.63%) having bee population stronger of 12400 to 13700 bees form Srinagar and Coimbatore colonies respectively. May be due to less amount of propolis collected by weak colonies to meet propolis demand they add beeswax to it.

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INTRODUCTION

Propolis is a resinous substance that bees collect from the exudates of plants and which they use to seal holes in the beehive (Marcucci *et al.*, 2001). Propolis, too, forms part of traditional medicine, and chemical analysis has pointed to the presence of at least 300 compounds in its composition (Castro 2001). It is mainly composed of resin (50%), wax (30%), essential oils (10%), pollen (5%), and other organic compounds (5%) (Gómez- Caravaca and others 2006). Among these organic compounds, we may find phenolic compounds and esters, flavonoids in all their forms (flavonoles, flavones, flavonones, dihydroflavonoles, and chalcones), terpenes, beta-steroids, aromatic aldehydes and alcohols, sesquiterpenes, and stilbene terpenes (Aga and others 1994; Russo and others 2002). As with honey, its composition varies with different factors, such as source of the exudates, climate, and environmental conditions (Chen and Wong 1996; Nieva-Moreno and others 1999). Caffeic acid phenethyl ester (CAPE) is a biologically active ingredient of propolis with several interesting biological properties, including apoptosis (Draganova-Filipova and others 2008), metastasis (Liao and others 2003), and radiation sensitivity (Chen and others 2005) of cancer cells. Propolis occurs in the form of a wax-like resin. Its melting point is usually in the 60–70 °C range, sometimes as high as 100 °C. Propolis consists mainly of resins (40–45%), waxes and fatty acids (25–35%), essential oils (10%),

pollen (5%), and organic compounds and minerals (5%) (Krell 1996). The composition varies according to geographic location and climate, since vegetation varies with environmental conditions. Propolis from Brazil has attracted the most interest because of its high biological activity.

MATERIALS AND METHODS

Collection and preparation of Propolis Samples

The crude Propolis Samples were collected from Jammu and Kashmir (Srinagar) and Tamil Nadu (Coimbatore) India during 2012 and 2013 respectively by Simple Scrapping method. Scrapped propolis samples were stored at -10 °C till processing.

Determination of impurities of propolis

For this purpose the known quantity of propolis (varying between 1-3 g) was weighed and mixed in known volume of 70 per cent ethyl alcohol. One part of propolis was crushed in nine parts of 70 per cent ethanol. This mixture was then placed in a close opaque dark coloured bottle and allowed to stand for 10 days for standardizing the period of extraction. The mixture was shaken two or three times a day. On the last day, the mixture was filtered through Whatman No. 01 filter paper with great care so as to obtain the extract. The filtrate was collected whereas, the residue was dried at room temperature and weighed. The residue represents the impurities (wax and mechanical impurities) present in the samples. By recording

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the differences in weight of propolis taken for extraction and the weight of residue, the percent solubility of propolis was determined. Impurities of propolis samples taken from colonies having different bee population were also determined in the same way.

Determination of impurities in different seasons

Same methodology were applied for different seasonal propolis collections which were pooled as Spring (March-April), Summer(May-June) and Autumn(July-August) to find out the percentage of wax and mechanical impurities.

Determination of impurities in different bee strength colonies

In the experiment, colonies of three different bee population viz, 5100-7300 (bees on 3-4 frames), 8900-10500 (5-6 frames) and 12400-13700 (7-8 frames) were used during August, 2012 and 2013. Respective numbers of bee colonies observed for this purpose were 15, 20 and 7 colonies. The traps (5 mesh double plastic net) were installed for one month and propolis collections were made to examine the Wax and mechanical impurities in relation with bee population.

RESULTS AND OBSERVATIONS

Wax and mechanical impurities in propolis

Wax and mechanical impurities in propolis collected from Srinagar (J&K) during 2012 and Coimbatore (TN) during 2013 in different month are depicted in Table 1, Fig. 1 and Table 2 Fig. 2 respectively. Impurities in the sample during March were to the extent of 62.60 and 63.13 per cent respectively. The impurities in samples of April were significantly less (52.30% and 53.13%) as compared to those of March month (62.60% and 63.13%) respectively. The samples collected during June to September had impurities in the range of 29.56 to 38.60 and 30.63 to 39.70 per cent from respective places differences being non-significant and these impurities ranges were at par with the samples of May months respectively. Samples collected in the month of May 2012 and 2013 from Srinagar and Coimbatore had 40.83 and 41.66 per cent impurities which were at par with samples of April but significantly less than those of March respectively. The percentage of mechanical impurities were found highest (62.60%) from Srinagar (J&K) and (63.13%) from Coimbatore (TN) in the month of March and lowest (29.56 and 30.63%) in the month of July respectively.

Table 1. Wax and Mechanical Impurities (%) in propolis samples collected from *Apis mellifera* colonies at Srinagar (J&K) during different months-2012

Month	Percent Wax and Mechanical Impurities(Extraction for 10 days)Srinagar(J&K)	SD \pm (SE \pm)
March	62.60	2.821(1.628)
April	52.30	3.251(1.877)
May	40.83	3.200(1.847)
June	30.66	3.401(1.964)
July	29.56	3.300(1.905)
August	38.60	3.300(1.905)
September	38.00	3.000(1.732)
Mean	41.79	
CD _{0.05}	8.88	

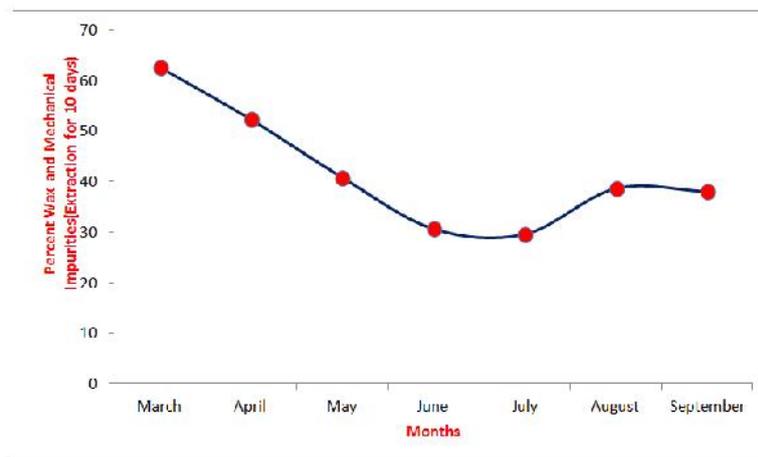


Fig. 1. Wax and Mechanical Impurities (%) in propolis samples collected from *Apis mellifera* colonies at Srinagar (J&K) during different months-2012

Table 2. Wax and Mechanical Impurities (%) in propolis samples collected from *Apis mellifera* colonies at Coimbatore(TN) during different months-2013

Month	Percent Wax and Mechanical Impurities(Extraction for 10 days) Coimbatore (TN)	SD \pm (SE \pm)
March	63.13	3.150(1.818)
April	53.13	3.150(1.818)
May	41.66	3.302(1.906)
June	31.83	3.002(1.733)
July	30.63	3.156(1.822)
August	39.70	3.104(1.792)
September	39.00	3.000(1.732)
Mean	42.72	
CD _{0.05}	8.713	

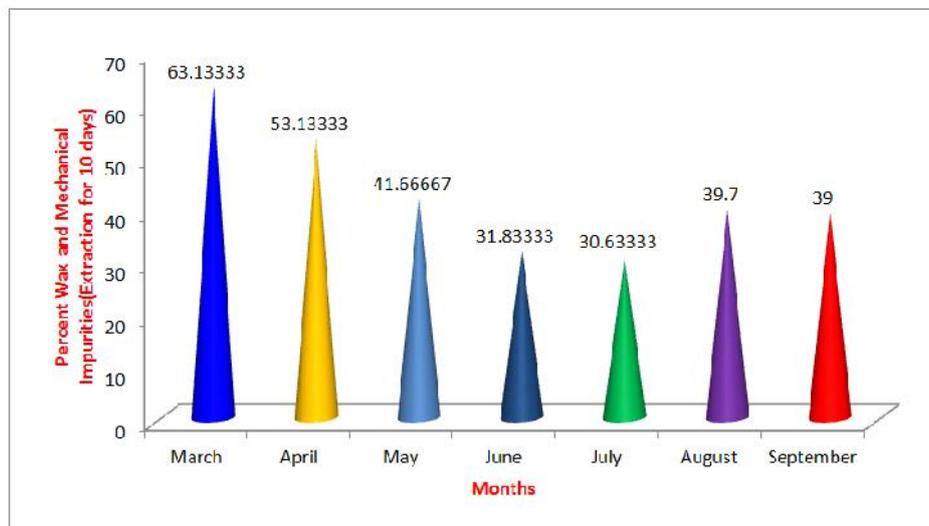


Fig. 2. Wax and Mechanical Impurities (%) in propolis samples collected from Apis mellifera colonies at Coimbatore (TN) during different months-2013

Wax and mechanical impurities in propolis during different seasons

Pooled data (Table 3,4 and Fig. 3,4) on impurities of propolis collected from Srinagar (J&K) and Coimbatore (TN) during 2012 and 2013 respectively revealed significant seasonal variations.

The propolis collected during summer and autumn season had significantly less impurities, respective values being 41.06, 42.26 and 34.00, 35.00 per cent than the sample collected during Spring 60.35 and 61.50 per cent

Table 3. Wax and Mechanical Impurities (%) in propolis samples collected from Apis mellifera colonies at Srinagar (J&K) during different seasons-2012

Season	Percent Wax and Mechanical Impurities(Ethanol Extraction for 10 days) Srinagar(J&K)	SD ± (SE ±)
Spring(March- April)	60.53	3.350(1.934)
Summer(May-June)	41.06	2.956(1.707)
Autumn (July-August)	34.00	3.000(1.732)
CD _{0.05}	7.781	

Table 4. Wax and Mechanical Impurities (%)in propolis samples collected from Apis mellifera colonies at Coimbatore (TN) during different seasons-2013

Season	Percent Wax and Mechanical Impurities(Ethanol Extraction for 10 days) Coimbatore (TN)	SD ± (SE ±)
Spring(March- April)	61.50	3.315(1.934)
Summer(May-June)	42.26	3.300(1.905)
Autumn (July-August)	35.00	3.000(1.732)
CD _{0.05}	8.098	

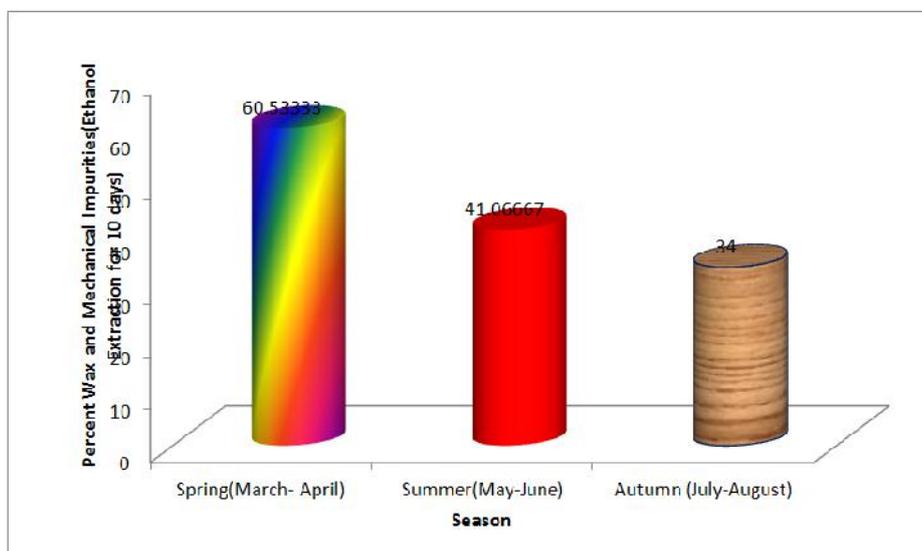


Fig. 3. Wax and Mechanical Impurities (%)in propolis samples collected from Apis mellifera colonies at Srinagar (J&K) during different seasons-2012

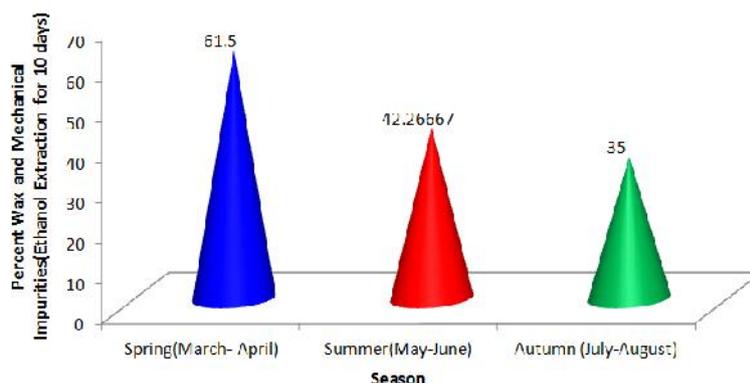


Fig. 4. Wax and Mechanical Impurities (%) in propolis samples collected from *Apis mellifera* colonies at Coimbatore (TN) during different seasons-2013

Effect of bee strength on wax and mechanical impurities

The observations made on level of impurities in the samples collected from colonies (Table 5 and 6, Fig. 5 and 6) revealed that colonies of different bee strength having low bee population (5100-7300) had maximum (66.60 and 68.03%) impurities.

With increase in bee population, the level of impurities declined significantly. The samples from colonies having bee population of 8900-10500 had 55.58 and 56.55 per cent impurities as compared to only 38.66 and 39.63 per cent impurities respectively in colonies having population of 12400-13700 bees from two respective regions Srinagar (J&K) and Coimbatore (TN) India during 2012 and 2013 respectively.

Table 5. Wax and Mechanical Impurities (%) in propolis samples collected from *Apis mellifera* colonies of different population at Srinagar (J&K) during months of August 2012

Bee Population in Hives	Percent Wax and Mechanical Impurities (Ethanol Extraction for 10 Days) Srinagar (J&K)	SD ± (SE ±)
5100-7300	66.60	3.451(1.992)
8900-10500	55.58	3.450(1.992)
12400-13700	38.66	3.455(1.995)
CD _{0.05}	8.649	

Table 6. Wax and Mechanical Impurities (%) in propolis samples collected from *Apis mellifera* colonies of different population at Coimbatore (TN) during months of August 2013

Bee Population in Hives	Percent Wax and Mechanical Impurities (Ethanol Extraction for 10 Days) Coimbatore (TN)	SD ± (SE ±)
5100-7300	68.03	2.715(1.567)
8900-10500	56.55	3.375(1.948)
12400-13700	39.63	3.380(1.951)
CD _{0.05}	7.946	

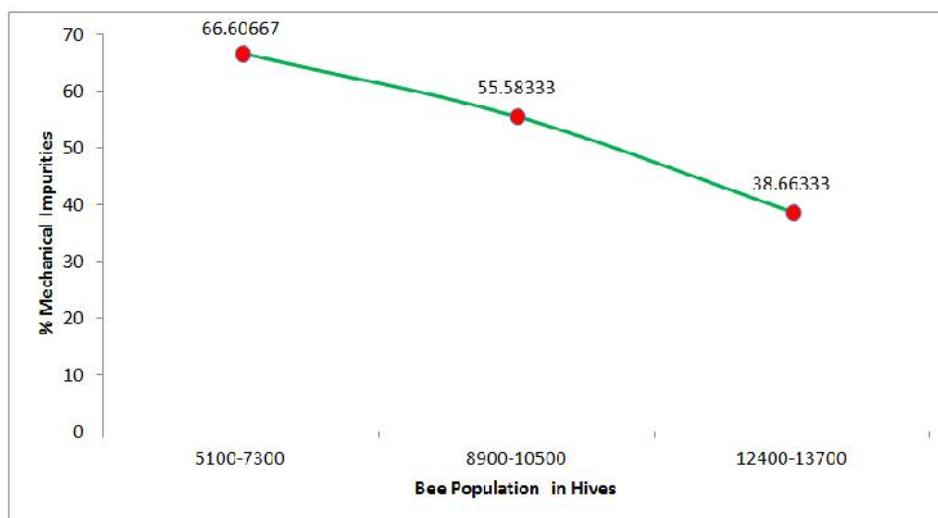


Fig. 5. Wax and Mechanical Impurities (%) in propolis samples collected from *Apis mellifera* colonies of different population at Srinagar (J&K) during months of August 2012

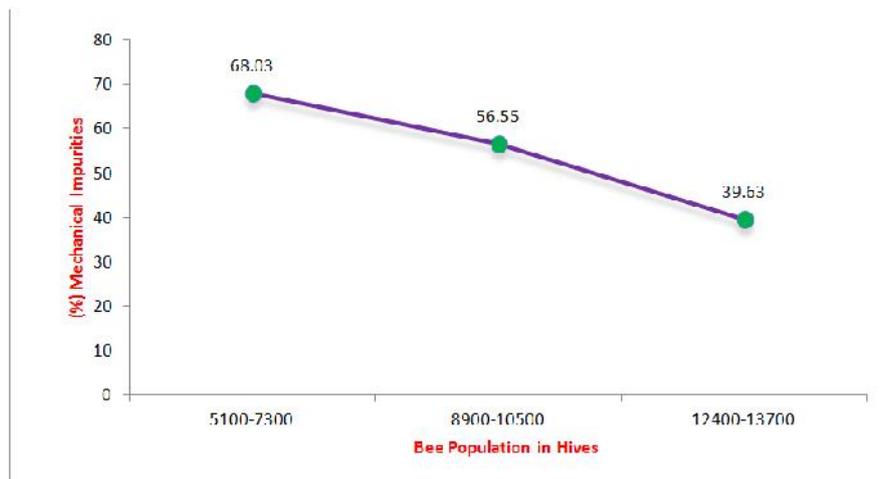


Fig. 6. Wax and Mechanical Impurities (%) in propolis samples collected from *Apis mellifera* colonies of different population at Coimbatore (TN) during months of August 2013

DISCUSSION

Impurities in propolis were more, when less propolis was collected by the bees from respective places during spring (60.53 and 61.50%) and Summer (41.06 and 42.26%) as compared to those of Autumn season (34.00 and 35.00 %) from Srinagar and Coimbatore respectively. Bratkowszki and Wilde (2002) have also made similar observation. It may be possible that during colder periods bees mix more wax to meet the colony demand of propolis. Impurities in propolis have been reported to vary even with strength of bee colonies from which the samples have been tested (Rybak *et al.* 1992). The observation made in the present study collaborate with these findings. Impurities in propolis sample from low bee population (5100-7300 bees/colony) were more (66.60 and 68.03%) as compared to the samples from stronger colonies (38.66 and 39.63%) having bee population stronger of 12400 to 13700 bees form Srinagar and Coimbatore colonies respectively. May be due to less amount of propolis collected by weak colonies to meet propolis demand they add beeswax to it.

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