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Chemical analysis of carbohydrates and neonicotenoid residue in honey collected from nearby districts of U.S. Nagar

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Abstract
In the recent years the growing demand for contaminant-free honey has increased the interest in the analysis part of its carbohydrate content and pesticide residue in order to ensure its quality. Thus present study was aimed to analyse the carbohydrate content and imidacloprid residue in the different honey samples collected from ten different locations in the district Udham Singh Nagar during 2015-16. Significantly higher carbohydrate contents *viz.* fructose, glucose, sucrose & maltose were observed in the sample collected from Dineshpur. Fructose per cent of honey samples was found ranging between 29.24 – 41.19%, Glucose per cent 21.78 -35.69%, Sucrose per cent 4.85 -17.93% and Maltose per cent 2.37%. Moreover all the samples were found to be free from Imidacloprid residue when analysed with HPLC technique. Thus it was concluded that Dineshpur is producing comparatively high quality honey whereas in the absence of imidacloprid residue in all honey samples, revealed that these are safe to consume.

Keywords: Fructose, glucose, sucrose, maltose, HPLC (with RI detector), HPLC-UV, Imidacloprid

1. Introduction
Human history reveals the importance of honey since its existence on earth as an important carbohydrate source and the only chiefly available oldest sweetener until industrial sugar production began to swap it after 1800. Honey is cited in the holy book of ancient India, the Rig Veda (1:90:6-8), ancient China, the Shi Jing, in The Bible it is mentioned that the first food given to Jesus Christ was fish and honeycomb, The Koran recommended honey as wholesome food and excellent medicine and many more. It is the natural sweet substance produced by honey bees. Honey bee collect nectar from plant-blossoms or from the secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which honey bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature [4]. Honey is primarily a carbohydrate product and make up as much as 99% of the total solids. Dextrose and levulose account for about 85 per-cent of the total carbohydrate content in honey. Two disaccharides *viz.* sucrose and maltose also found in honey but in low levels. Moreover, 22 other sugars have also been found, which are more complex than these monosaccharides dextrose and levulose. Thus the quality of honey depend on the sum of fructose, glucose, fructose/glucose ratio and glucose/water ratio [7]. Fructose/glucose ratio indicates the ability of honey to crystallize [3]. The presence of these natural sugars in honey makes it highly nutritious. But this carbohydrate composition of honey could vary with several biotic and abiotic factors *viz*. geographical area, type of flora, prevailing season, environmental factors and treatment of beekeepers [6]. Moreover, honey also comprises various medicinal properties. These all unique properties of honey makes it much costly than other sweeteners and simultaneously susceptible to adulteration with cheaper sweeteners, predominantly sucrose. Thus recently growing demand for contaminant-free honey products has increased the interest in the analysis part of its carbohydrate content in order to ensure its quality.
In addition pesticides which, has caused several environmental problems also leading to the residue problem in honey, which constitutes a potential risk for human health [5]. Pesticide application in crops can contaminate soil, air, water, and the flowers from which bees collect nectar for honey production. Hives could be contaminated by direct or indirect exposure. Therefore, the determination of contaminants and residues in honey and other bee products has become a growing concern in recent years, especially as these compounds may diminish the

beneficial properties of honey and, if present in significant amounts, may pose a serious threat to human health. Thus the purpose of the current scientific study was to monitor the carbohydrate content and pesticide residues in honey samples collected from different locations of U.S. Nagar (Uttarakhand), which will help to ensure the consumer health protection, national and international commercial competition, and better product quality of honey.

2. Materials and Methods

The present analysis was carried out at laboratory available in G. B. Pant University of Agriculture and Technology, Pantnagar-263145, District: Udham Singh Nagar (Uttarakhand) India.

2.1. Experimental design and details of treatments

The experiment was laid out in CRD (Completely Randomized Design) that was consisted of ten treatments with five replications of each. Honey samples were collected from ten different locations in Udham Singh Nagar *viz.* Dineshpur, Ramnagar, Tanda, khatima, Rampur, Almora, Pantnagar, Haldwani, Halduchaor, and Pilibhit. Collected samples were stored at ambient temperature until analysed. Imidacloprid was taken into consideration for residue analysis in the honey samples. The numerical data of all the components were subjected to analysis of variance (ANOVA) using CRD to calculate critical difference (CD) using standard statistical procedure.

2.2. HPLC method for carbohydrate and residue analysis:

Each honey sample was analysed for fructose, glucose, sucrose, maltose using HPLC (High Performance Liquid Chromatography) equipped with RI- Detector and imidachloprid residue using HPLC equipped with UV detector.

2.3. Operating Conditions

HPLC method for carbohydrate and residue analysis

Particulars	Carbohydrate analysis	Residue analysis
Mobile Phase	85% Acetonitrile / 15% H_2O degassed	75% Acetonitrile / 25% H_2O degassed (600 ml)
Column	Amino column	Amino column
Pump	Pmax - 3,000 -3200 psi	34 kgf
Flow Rate	1.5 ml/min	0.8 ml/min
Detector	Room temperature	Room temperature
Syringe Volume	250 µL	250 µL
Rheodyne Injection Loop	20µL (actual volume injected in HPLC)	20µL (actual volume injected in HPLC)
UV Absorbance/RI detector	RI- Detector	230 nm
Oven Temperature	-	30 ^{0}C

2.4. Extraction procedure

50g of honey sample was weighed in a flask. The sample was mixed with 5 ml of water and homogenized by shaking to reduce its viscosity and to facilitate handling. The sample was mixed with 50 ml of solvent (ethyl acetate) and was submitted to extraction by agitating for 20 min. In a separator funnel the organic phase was separated by centrifugation at 2500 rpm for 10 min. The supernatant was collected and the residues were re-extracted with 40 ml of solvent. The solvent was evaporated in rotary evaporator under reduced pressure at 65 °C. Finally the residues were dissolved in 5 ml of ethyl acetate and passed through a 0.50 µm sized pore PTFE filter. The samples were then cleaned by adding 0.5 g silica gel, 1g anhydrous sodium sulphate, 5g mixture of activated carbon and silica gel or florisil. These were then passed through a chromatographic column and then the filtered extracts received. Then 1 ml of ethyl acetate was added to this eluate and was submitted to analysis by HPLC-UV.

3. Result and Discussion

The data pertaining to Fructose, glucose, sucrose and maltose per cent in honey samples collected from nearby districts of U.S. Nagar are presented in Table 1. Figures show the HPLC chromatograms of standards and honey samples.

3.1. Fructose

In the current analysis the fructose per cent in different samples of honey was calculated with the help of calibration graph shown in Fig-1 of fructose standard**.** Analysis revealed the fructose per cent ranges between 29.24 – 41.19%. Significantly higher Fructose per cent was observed in honey sample of Dineshpur (41.19%). However, sample collected from Halduchaor (36.86%), khatima (36.20%), Almora (35.52%) Tanda (34.44%) did not differ significantly in fructose per cent. The fructose content in the samples collected from Rampur (30.16%), Haldwani (29.99%) and Pantnagar (29.24%) was at par with each other. Lowest fructose percent was obtained in honey sample collected from Pantnagar (29.24%). The average of fructose per cent was 33.67%. The fructose content in honey samples collected from Ramnagar, Rampur, Pantnagar, Pilibhit and Haldwani was found below mean value. Similar results were also reported by **[3]** who stated that the fructose per cent should be between 37.68 to 40.31%.

3.2. Glucose

In the present investigation the glucose per cent in different samples of honey was calculated with the help of standard calibration graph shown in Fig-2 and chromatogram of glucose in respective sample obtained through HPLC. Its per cent was reported ranging between 21.78 -35.69%. Significantly higher Glucose per cent was obtained in honey sample collected from Dineshpur (35.69%) while the samples collected from Almora (33.48%) and Halduchaor (33.09%) were at par with each other. Lowest glucose percent was found in honey sample brought from Pilibhit (21.78%). The average value of glucose per cent was 29.29%. Honey samples collected from Ramnagar, Rampur, Pantnagar, Pilibhit and Haldwani were below mean glucose value. According to [3] the glucose% should be between 27.25 to 39.56%. Whereas [1] reported that glucose per cent in the range of 19.6 - 27.6%.

3.3. Sucrose

In the present study the sucrose per cent in different collected samples of honey was calculated with the help of calibration graph shown in Fig-3 of sucrose standard**.** The per cent of sucrose was found ranging between 4.85 -17.93%. Significantly higher sucrose per cent was observed in honey sample of Dineshpur (17.93%) followed by khatima (14.36%), Almora (10.10%), Haldwani(8.52%) and Ramnagar (7.83%). Lowest sucrose percent was found in honey sample brought from Halduchaor (4.85%). However, no sucrose content was found in the sample collected from Tanda, Rampur, Pantnagar and Pilibhit. The average value of sucrose content in the samples was found 6.36%. The average content of sucrose in honey samples were in accordance with the findings of [7].

3.4. Maltose

The maltose per cent in different samples of honey was calculated with the help of calibration graph shown in Fig-4 of fructose standard. Maltose content was reported only in the sample collected from Dineshpur 2.37%, however remaining honey samples were not detected with the maltose content.

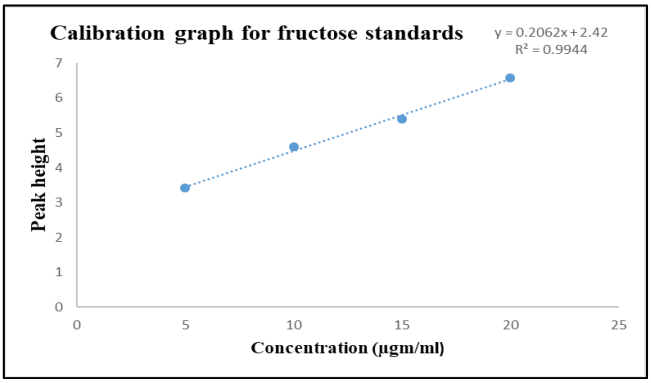

Fig 1: Standard calibration graph for Fructose

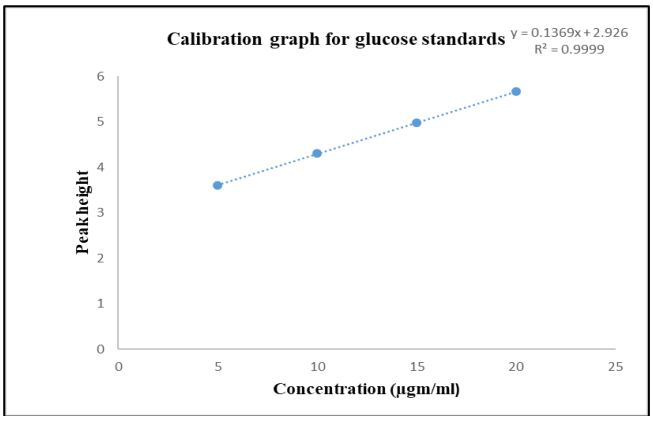

Fig 2: Standard calibration graph for Glucose

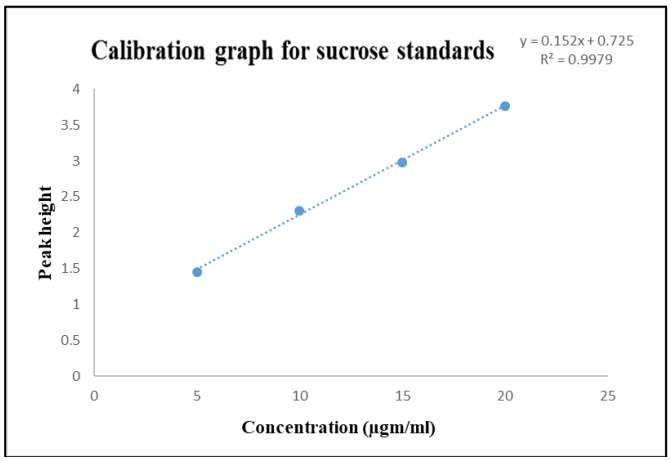

Fig 3: Standard calibration graph for sucrose

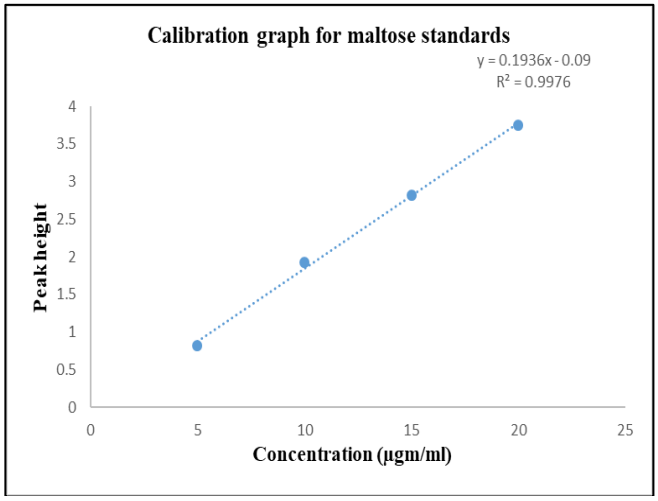

Fig 4: Standard calibration graph for Maltose

Table 1: Determination of Fructose, Glucose, Sucrose and Maltose Concentrations in Honey Samples collected from nearby areas of U.S. Nagar.

Treatments	Fructose (%)	Glucose (%)	Sucrose (%)	Maltose (%)
T_1	41.19	35.69	17.93	2.37
T_2	32.34	28.41	7.83	0.0
T_3	34.44	30.20	0.0	0.0
T_4	36.20	32.20	14.36	0.0
T_5	30.16	27.31	0.0	0.0
T_6	35.52	33.48	10.10	0.0
T_7	29.24	26.54	0.0	0.0
T_8	29.99	24.21	8.52	0.0
T_9	36.86	33.09	4.85	0.0
T_{10}	30.79	21.78	0.0	0.0
Mean	33.67	29.29	6.36	0.23
SEm	1.04	0.43	0.12	0.027
CD at 5%	2.99	1.23	0.37	0. 078

T_1-Dineshpur, T_2-Ramnagar, T_3-Tanda, T_4-Khatima, T_5-Rampur, T_6-Almora, T_7-Pantnagar, T_8-Haldwani, T_9-Halduchaor, T_{10}-Pilibhit

3.5. Imidacloprid Residue

The standard calibration graph and HPLC chromatogram of imidacloprid is presented in Fig-5 and Fig-6, respectively. The fraction revealed 1 major peak with 3.65 retention time (RT) in the range of 3.5 – 3.9 min for 20 µL injection volume. By calculation it was found that no residue is present in all tested honey samples of different locations, when compared with Imidacloprid sample, used as standard, Fig-7. The results revealed that there is no contamination of Imidacloprid residue in honey samples of 10 different locations of nearby areas of U.S. Nagar.

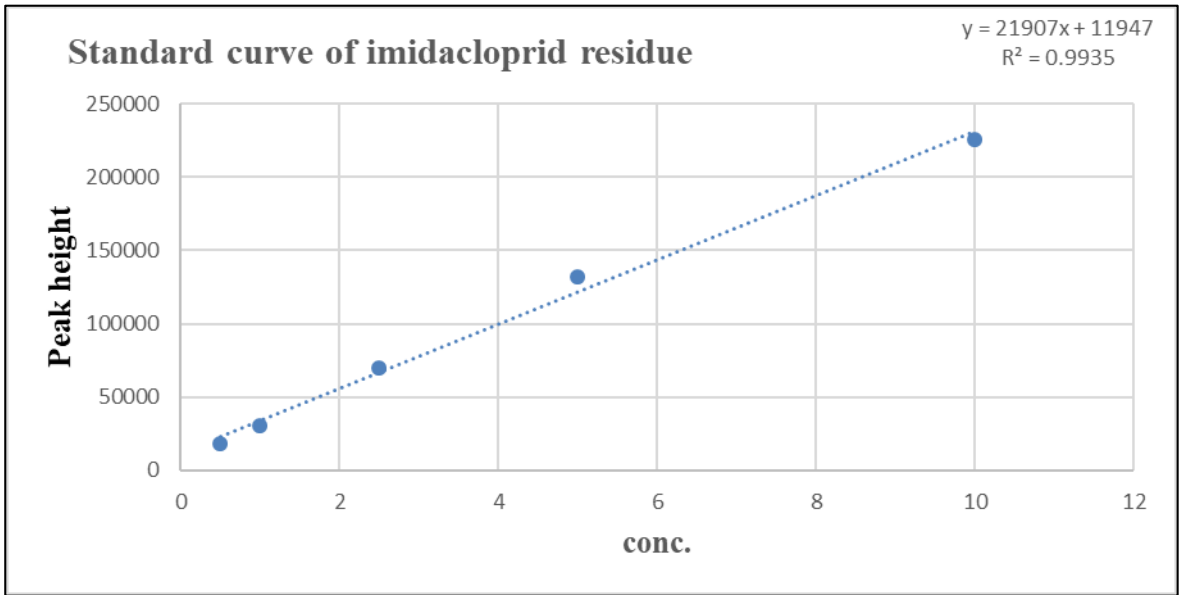

Fig 5: Standard Calibration curve of Imidacloprid residue

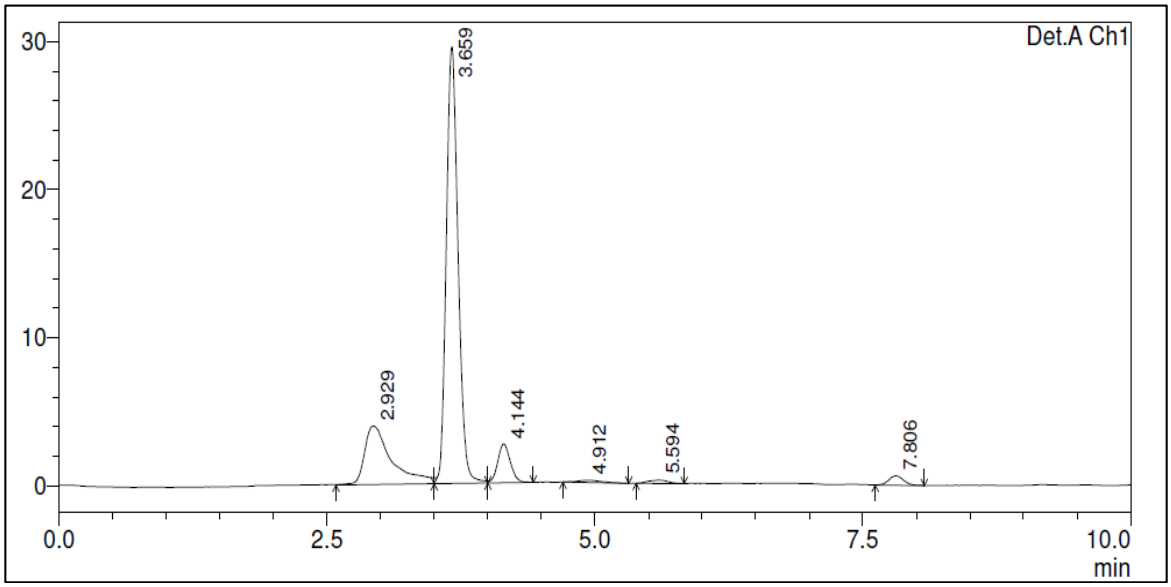

Fig 6: Chromatogram of Imidacloprid Standard by HPLC-UV

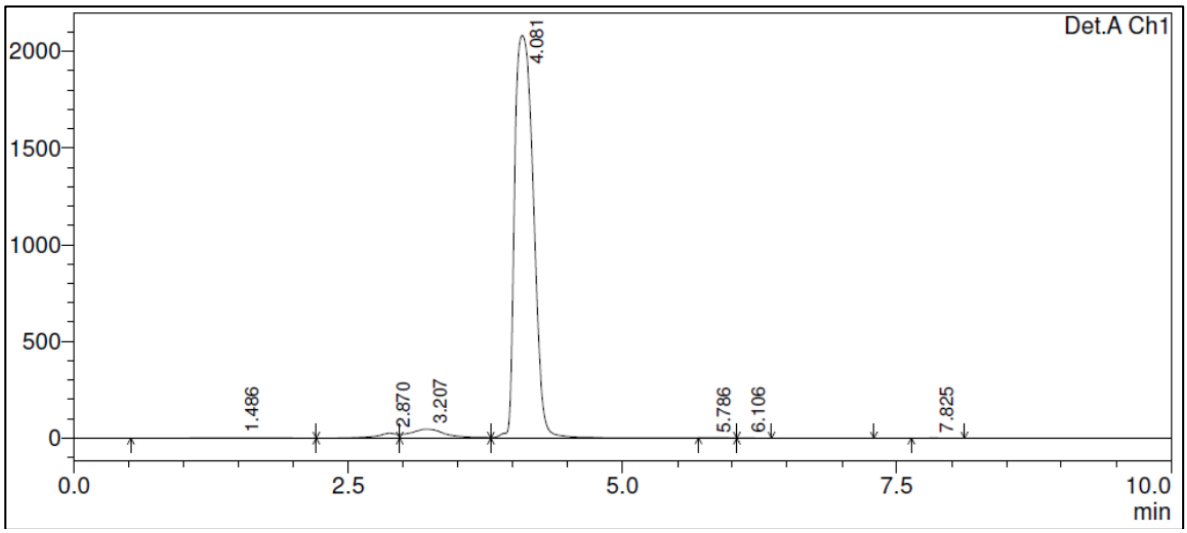

Fig 7: Chromatogram of honey sample by HPLC-UV

4. Conclusion

In the current study it was concluded that among all honey samples the highest fructose content (in ideal range) was found in honey sample collected from Dineshpur (T1) thus ensuring for producing comparatively more quality honey while relatively lowest fructose content was found in sample collected from Pantnagar. Likewise, the lowest glucose per cent was reported in Pilibhit honey sample, in the honey samples collected from Tanda (T3), Rampur (T5), Pantnagar (T7) and Pilibhit (T10) sucrose was not detected, while maltose(in small amount) was detected only in the sample collected from Dineshpur. In addition, it was found that the predominant sugar in all ten investigated honey samples was fructose followed by glucose, while sucrose and maltose were recorded in low quantity in all collected samples. Thus the study has ensured that the honey sample of Dineshpur is comparatively high quality honey. Moreover, it was found that none of the samples contain residue of Imidacloprid, which reveals that these collected honey samples are safe to consume.

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6. References

1. Ahmad A, Azim MK, Mesaik MA, Khan RA. Natural honey modulates physiological glycemic response compared to simulated honey and D-glucose. Journal of Food Science. 2008; 73:165-167.
2. Amir Y, Yesli A, Bengana M, Sadoudi R, Amrouche T. Physico-chemical and microbiological assessment of honey from Algeria. Electronic Journal of Environmental Agriculture and Food Chemistry. 2010; 9:1485-1494.
3. Buba F, Gidado A, Shugaba A. Analysis of biochemical composition of honey samples from North-East Nigeria. Biochemistry and Analytical Biochemistry. 2013; 2(3):139.
4. Codex Alimentarius Commission Codex Standard for Honey, FAO, Rome. Alinorm. 2001a; 1:19-26.
5. Colin ME, Bonmatin M, Moineau I, Gaimonc, Brun S, Vermandere JP. A method to quantify and analyze the foraging activity of honey bees: relevance to the sublethal effects induced by systemic insecticides. Archives of Environmental Contamination and Toxicology. 2004; 47(3):387.
6. EL-Metwally AAE. Factors Affecting the Physical and Chemical Characteristics of Egyptian Beehoney. Ph. D. Thesis, Faculty of Agriculture, Cairo University, 2015, 320.
7. Kaskoniene V, Venskutonis PR, Eksteryte V. Carbohydrate composition and electrical conductivity of different origin honeys from Lithuania. LWT – Food Science and Technology. 2010; 43:801-807.