NEW TECHNICAL PROGRAMME

Department of Forest Products and Utilization

Experiment No. 21.5.3.10

01.	Experiment No. and Title	:	**Comparative evaluation of different analytical methods for the quantitative analysis of micronutrients in honey**
02.	Budget Head	:	12101, 12952 & 12019
03.	Collaborative department, if any	:	-
04.	Background information		
	Honey is a complex natural product valued for its nutritional and medicinal properties. It contains essential and trace elements that influence its quality, authenticity, and safety. Elemental analysis of honey is crucial for detecting beneficial minerals and potential contaminants that may originate from environmental pollution, soil composition, or processing methods. Various analytical techniques, such as Inductively Coupled Plasma - Optical Emission Spectroscopy (ICP-OES) and Atomic Absorption Spectroscopy (AAS) are employed to assess these elements. However, number of digestion techniques is commonly used for elemental analysis from honey samples *viz.* Wet digestion with diacid (HNO_3:$HCLO_4$), Diacid digestion after ashing at 550 ^{0}C, Wet digestion with HNO_3 plus H_2O_2. Considering complex nature of honey matrix and practical feasibility in terms of cost, duration, ruggedness in analytical performance, there is need to identify most suitable digestion method and instrument technique to determine the elemental composition in honey. Therefore, the proposed study aims to find out the suitable digestion method along with instrumental technique to quantify elemental composition of honey. The information generated can be useful to determine the elemental composition of honey with higher degree of confidence and it could further be exploited for ensuring food safety, regulatory compliance in respect to honey."		
	Hypothesis	:	**Null Hypothesis (H0)** There is no significant difference in the elemental composition of honey samples analyzed using different digestion techniques (Wet digestion with diacid, Diacid digestion after ashing at 550 °C, Wet digestion with HNO_3 plus H_2O_2) when measured by ICP-OES and AAS. **Alternative Hypothesis (H1)** There is a significant difference in the elemental composition of honey samples analyzed using different digestion techniques (Wet digestion with diacid, Diacid digestion after ashing at 550 °C, Wet digestion with HNO_3 plus H_2O_2) when measured by ICP-OES and AAS.
05.	Objectives	:	1. To standardize the quantitative analysis of micronutrients in honey with analytical methods using different digestion techniques

				2. To assess the comparative efficiency of different analytical methods employed for determining micronutrients in honey
06.	Principal investigator and associates		:	**PI:** Dr**.** D. P. Patel, Assoc**.** Professor (NRM) **Co-PIs:** Dr. A. A. Mehta, Assoc. Professor (FPU) Dr. S. V. Viyol, Assist. Res. Scientist (Env. Sci.) **Associate scientist:** Dr. Susheel Singh, Asst. Professor (Reside Chemistry), FQTL, N M College of Agriculture **RA/SRF:** Dr. Govind Dr. Ram Mevada
07.	Location and Agro-climatic sub-region		:	NA
08.	Year of commencement		:	2025-26
09.	Tree species		:	NA
	Experimental details		:	
	(a)	Treatments	:	**Factor (A): Digestion with acids** D_1: Wet digestion with diacid (HNO_3:$HCLO_4$) D_2: Diacid digestion after ashing at 550 ^{0}C D_3: Wet digestion with HNO_3 plus H_2O_2 **Factor (B): Quantification with instruments** M_1: ICP-OES M_2: AAS
	(b)	Experimental Design	:	CRD and Descriptive statistics
	(c)	Repetition	:	3
	(d)	Plot size (if applicable)	:	NA
	(e)	Spacing	:	NA
	(f)	Seed rate (kg/ha)	:	NA
	(g)	Manures and fertilizer	:	NA
	(h)	Any other detail, if required	:	NA
10.	Observations to be recorded		:	1. **Concentration of microelements (mg/kg):** Fe, Mn, Zn, Cu, Ni in accordance with treatment combinations 2. **Standardization of methods** (a) Specificity (b) Linearity (c) Accuracy (% recovery) (d) LOD and LOQ (e) Precision (% RSD) 3. **Economic analysis** (a) Cost of analysis

			(b) Time of analysis
11.	Methodology	:	Prior to analyze the honey samples, the analytical methods will be standardized (ICH, 2007) by determining linearity, LOD and LOQ, accuracy and precision. After standardization, the honey samples will be subjected to different digestion and instrument techniques and most efficient methodology in terms of easiness, accuracy, cost and time will be adopted for real sample analysis. **Following points will be considered for performing the present experiment** **1.** Honey will be collected from different sources *viz.*, *Apis mellifera*, *A. cerana* and *Tetragonula* spp. (Stingless bee) **2.** Recovery study will be carried out at level X, 5X and 10X of LOQ **3.** Percent RSD of recovery at different fortification levels will be used to assess precision **4.** Specificity will be determined with spiked samples/ matrix blanks **5.** To determine the Limit of Detection (LOD) and Limit of Quantitation (LOQ), calibration curve approach will be used and calculating them based on the standard deviation of the response (σ) and the slope of the calibration curve (S) using the formulas: LOD = $3.3\sigma/S$ and LOQ = $10\sigma/S$ **6.** Linearity will be assessed by preparing a series of calibration standards with known analyte concentrations, analyzing them, and plotting the results against the expected concentrations. Then, statistical analysis, such as linear regression will be performed to assess the relationship and determine if the method exhibits a linear response within the tested range.