

# Chemical Composition Of Honey: A Dual Approach To Assess Pesticide Presence And Quality From Nashik District, Maharashtra

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## Abstract-

Honey is a natural, nutrient- rich sweetener that offers many health advantages, making it a great choice for everyday meals. The chemical composition of honey plays a crucial role in determining its quality, authenticity, and health benefits. In India, the demand for honey is growing, with an average yearly consumption of 50- 150 grams per person, accounting for 3-5% of the world's total honey usage. To increase profits, honey is frequently mixed with artificial additives, reducing its health benefits and potentially causing diabetes, toxicity or allergic reactions. It is very important to study the chemical components of honey from natural honey combs. This study investigates the chemical properties of honey, focusing on the identification of key compounds such as sugars, acids, proteins, enzymes, and trace elements that define its unique characteristics. Pesticide residues in food can pose the serious health hazards. The detection of pesticide residues in honey has become an increasing concern due to potential health risks and its implications for food safety and environmental protection. Therefore, studying pesticide contamination in honey is of great importance. This research investigates the presence of pesticide residue in honey samples from various regions of in the Nashik district of Maharashtra. Advanced analytical methods, such as Gas chromatography- Mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC), were employed to identify the chemical components of honey from different floral sources. The results indicate pesticide contamination in honey from areas with significant agricultural development. This study enhances our understanding of honey's chemical composition, shedding light on its nutritional value. Additionally, it assesses the impact of pesticide contamination in honey from agricultural regions and emphasizes the need for improved testing protocol to protect consumers and promote more sustainable farming practices.

**Keywords-** Honey, Analysis, GCMS, Pesticides, Sugars, Amino acids, Enzymes, chemical composition

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## INTRODUCTION

Honey, created by honeybees, is a natural sugar solution that is supersaturated, offering high nutritional value. It consists of a complex blend of carbohydrates and is widely regarded as the only concentrated form of sugar available globally. Honey is a highly complex natural food, containing around 200 different substances (da Silva et al., 2016; Pita-Calvo et al., 2017), with carbohydrates, particularly reducing sugars like fructose and glucose, being the primary components (Trifković, et al., 2017). In addition to sugars, honey also includes proteins and amino acids, lipids, vitamins, phenolic compounds, minerals, and organic acids, among other substances (Afrin et al., 2020; Rahman et al., 2017; Trifković et al., 2017). Honey is widely used around the world, not only as a natural sweetener but also for its medicinal, nutritional, and cosmetic benefits. The ways in which honey is consumed and utilized differ significantly between countries due to cultural preferences, availability, and traditional practices. The global production of honey is nearly 18,000 metric tons, with China being the largest producer, accounting for 28% of the total production. Other major producers include Turkey (5.9%), Iran (4.5%), and the United States (4.1%). India ranks 6th, contributing 3.5% to global production (Singh, R., 2021). China is the largest producer and consumer of honey globally, responsible for about 25-30% of global consumption and producing nearly a quarter of the world's honey (FAO, 2021). On average, each person in China consumes 300-500 grams of honey annually. It is widely used in Chinese food, traditional medicine, and skincare (Gao, J., et al., 2019). The United States is a significant consumer of honey but relies on imports due to limited domestic production. Americans consume an average of 1.1 kg per person each year, contributing to 15-20% of global consumption (USDA, 2020). Honey is often used as a sweetener and in health-related products. Turkey is known for its high-quality honey, with per capita consumption ranging from 1-1.5 kg annually, accounting for 5-6% of global consumption. Germany also consumes about 1.5 kg per person per year, representing 7-10% of

the global market (German Honey Association, 2020). In India, honey consumption is on the rise, with an average per capita intake of 50-150 grams annually, making up 3-5% of global consumption (Naik, R. S., et al., 2015). Greece, where thyme honey is popular, has a per capita consumption of approximately 1 kg per person each year, contributing 2-3% of global consumption (Bontempo, P., et al., 2018). Other countries such as France, Italy, the UK, and Australia have consumption rates of 500-700 grams per person per year, while Mexico averages 300-400 grams. In Japan, consumption is steadily increasing, with an estimated 200-300 grams per person per year, representing 2-3% of global honey consumption (Japanese Honey Industry Report, 2020). Overall, honey consumption is growing around the world, especially in countries like India, Mexico, and Japan, as more people turn to natural sweeteners and seek the health benefits of honey.

Honey is often mixed with cheaper or artificial ingredients to boost volume and cut down on manufacturing expenses. Adulterated honey loses its natural nutrients while gaining health risks. The added sugars may promote diabetes and obesity, synthetic additives could introduce toxins, and unknown ingredients might trigger allergies, making impure honey a potential hazard. It is imperative to evaluate the chemical components of honey from hives.

The connection between honey's composition and its source is significant, as it has been used for many years as a method to determine honey authenticity (Trifković et al., 2017). The biochemical properties and quality of honey are influenced by factors such as its maturity, the climate in which it is produced, the methods of production, processing, and storage, as well as the type of nectar source used by the bees (White Jr JW 1978, Crane E. 1979, Bogdanov S. 1999, Oddo LP and Bogdanov S. 2004, Guler A. et al. 2007). The composition of these elements in honey is determined by the type of raw floral materials collected by the bees, including nectar, pollen, and honeydew (Baroni MV et al. 2009, Nanda V et al. 2009, Juszczak L et al. 2009, Silva LR et al. 2009). Furthermore, analysing the composition of honey is important for quality control, ensuring the presence of compounds that contribute to its health benefits and nutritional value (Pita-Calvo et al., 2017; Pušćion-Jakubik et al., 2020). Recently, plant microribonucleic acids (miRNAs), which play a key role in regulating gene expression in cells, have been detected in honey (Gismondi et al., 2017; Smith et al., 2021). These miRNAs could offer valuable insights in the future, potentially helping to explain some of honey's biological properties.

Honey, with its high carbohydrate content, serves as an excellent source of energy and offers several functional benefits. It is particularly beneficial for children and athletes. The glycemic index of honey ranges from 32 to 91, depending on its botanical origin (Bogdanov S., 2010). In addition to carbohydrates, honey contains a variety of other constituents in small and trace amounts. This diverse composition contributes to numerous nutritional and biological effects, including antimicrobial, antioxidant, prebiotic, and immunomodulatory properties. Recommendations on consumption rates have also been suggested.

Nashik, located in the state of Maharashtra, India, is known for its thriving agricultural sector due to its favourable climate, soil conditions, and irrigation facilities. The region's agriculture is diverse, supporting a wide range of crops, including fruits, vegetables, and grains. Over time, Nashik has embraced modern agricultural techniques, including the use of hybrid seeds, advanced fertilizers, and pesticides, to enhance crop yields and ensure plant health. Pesticide contamination in human life refers to the presence of pesticide residues in food, water, air and soil, all of which can pose risks to human health. This contamination can occur through various routes, such as agricultural practices, improper disposal, and exposure during pesticide application. The effects can be both immediate (acute) and long-term (chronic), health risks depending on the type and amount of pesticide as well as the frequency and duration of exposure. Bees can unknowingly collect pesticide residues while gathering nectar from recently treated flowers or nearby crops. These chemicals can be brought back to the hive and potentially end up in the honey. Additionally, pesticides can sometimes drift through the air, exposing bees to residues that may accumulate in honey production.

Pesticide analysis in food is crucial for safeguarding public health especially for vulnerable populations-ensuring regulatory compliance, maintaining trade integrity, preventing economic losses, supporting sustainable agriculture, protecting ecosystems from contamination. Examining pesticide residue in honey is particularly important as honey consumption increases among people. This research also emphasizes pesticide analysis due to the growing use of honey in people's diets.

Various approaches, from physical sensors to bioindicator organisms, are employed for environmental surveillance. *Apis mellifera*, the European honeybee, is a widely managed pollinator capable of

functioning as an ongoing biological monitor. As they forage, these bees encounter and accumulate environmental pollutants and pathogens, transporting them back to their colonies where analytical detection becomes possible (Cunningham, M. M. et Al., 2022).

**Materials and methodology-**

**Sample collection-** From Nashik district of Maharashtra, honey samples were collected from different locations. Collected honey samples were stored in airtight containers (glass jars) in cool and dry place at room temperature till bio estimation. Honey samples from the wild areas sample 1 from Wangan-Sule of Surgana tehsil, sample 2 from Tringalwadi of Igatpuri tehsil and agricultural areas- Sample 3 from Satana town of Satana tehsil, Sample 4 from Kasabe-Sukene of Niphad tehsil were analyzed to study the contents. For pesticide estimation, Gas Chromatography-mass Spectrometry (GC-MS) was used.

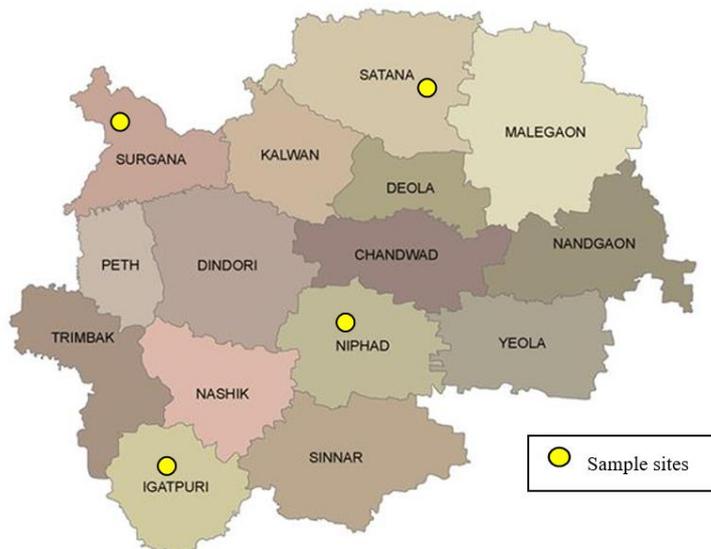


Image Source (Map)- <https://nashik.gov.in/about-district/map-of-district/>

Map 1: Showing sample sites in tehsil map of Nashik district, Maharashtra

Sr. No.	Sample Site	Tehsil	Latitude & Longitude	Type of Landform
1	Wangan Sule	Surgana	20.5357°N, 73.5021° E	Hilly
2	Tringalwadi	Igatpuri	19.7307° N, 73.5535° E	Forest (Hilly)
3	Satana	Satana	20.5982° N, 74.2033° E	Agriculture (Highly advanced)
4	Kasabe-Sukene	Niphad	20.0784° N, 74.0139° E	Agriculture (Highly advanced)

Table 1 Sites of honey samples

**Chemical analysis of honey samples-**

The enzymatic oxidation method using glucose oxidase was employed to measure glucose content in the honey samples. To quantify fructose content in honey, the resorcinol reagent method was used. Sucrose content was determined by inversion method (Layne, E. A. 1957; Boesel, P. A., & Galgano, F. L. 1987; AACC 2000). Finally, the Layne-Enyon method was applied and the sucrose content was obtained by difference. The inversion method (based on hydrolysis of sucrose followed by titration with Benedict's or Fehling's reagent) was used to estimate maltose concentration in honey. The vitamin C (ascorbic acid) content in honey samples was determined using the 2,6-dichlorophenolindophenol titrimetric method. DNS colorimetric assay was used to measure reducing sugars, which reflects invertase activity by quantifying glucose and fructose produced from sucrose hydrolysis. To estimate amylase concentration in honey, colorimetric assay for estimating amylase activity (modified of the DNS method) method was used. After measuring the absorbance of the honey sample, compared it to the standard curve to determine the concentration of reducing sugars, which reflects amylase activity. For determination of Amino acid, HPLC was used. To estimate the pesticides, GCMS was used. A Perkin Elmer Clarus 600C Gas Chromatograph

and Mass Spectrometer system with a GS-BP MS DB-5 capillary column was used for Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The components were identified by comparing their mass spectra to the Wiley 6.0 and NIST libraries.

## RESULTS -

### Biochemical Composition and Pesticide Residue Analysis of Honey Samples

#### 1. Sugar Content

The sugar profile of honey is crucial for determining its sweetness, nutritional value, and overall quality. In this study, four honey samples from the were analyzed for the levels of fructose, glucose, sucrose, and maltose.

- Fructose content ranged from 37% to 40.38%, with honey from Satana town of Satana tehsil showing the highest concentration at 40.38%, and Kasabe Sukene of Niphad tehsil honey shows the lowest at 37%. Fructose is a major sugar in honey, contributing significantly to its sweetness and caloric value.
- Glucose levels varied between 30% in honey sample from Satana town of Satana tehsil to 33.92% in honey sample from Wangan Sule of Surgana tehsil. The observed differences may reflect variations in floral sources.
- Sucrose concentrations were consistently low across all samples, ranging from 1.35% in honey from Wangan Sule of Surgana tehsil to 2.46% in honey from Satana town of Satana tehsil. Sucrose is usually found in minor amounts in honey.
- Maltose levels ranged from 1.64% in honey from Kasabe Sukene of Niphad tehsil to 2.98% in honey from Wangan Sule of Surgana tehsil. Maltose is a disaccharide formed from the breakdown of starches in nectar.

#### 2. Enzyme Activity

The activity of enzymes in honey is indicative of its freshness and quality. In this study, invertase and amylase were measured.

- Invertase activity ranged from 14 to 18 mg/100g across the samples. This enzyme is crucial for the breakdown of sucrose into fructose and glucose. The small variations in invertase levels across the samples might be attributed to differences in floral sources or environmental factors during honey production. Invertase activity is typically measured by assessing the release of reducing sugars (glucose and fructose) during the hydrolysis of sucrose by invertase.
- Amylase activity, expressed as dextrose equivalent, ranged from 55 to 62 D.E./100g, with honey from Satana town of Satana tehsil having the highest value at 62 D.E./100g. Amylase breaks down starches into simple sugars and its presence reflects both the nectar source and the enzymatic processes occurring during honey production. Amylase activity is determined by hydrolyzing starch into reducing sugars, following a process similar to that used for invertase.

Biochemical Tests	Biochemicals	Sample 1	Sample 2	Sample 3	Sample 4
Estimation of Sugars	Fructose (in %)	38	38.5	40.38	37
	Glucose (in %)	33.92	31.78	30	32.85
	Sucrose (in %)	1.35	1.42	2.46	1.98
	Maltose (in %)	2.98	2.21	2.47	1.64
Estimation of Enzymes	Invertase (mg/100g)	17	14	18	16
	Amylase (Dextrose equivalent/100g)	55	59	62	58
Estimation of Vitamin C	Ascorbic acid (mg/100g)	21	19	20.5	22
Estimation of Amino acids	Proline(mg/100g)	52	54	49	57
	Valine(mg/100g)	4	6	8	5.5

pH		3.8	3.9	4.1	3.87
Pesticides (Using GCMS)		Not Detected	Not Detected	Detected	Detected

Table 2 Biochemical analysis of honey samples

### 3. Vitamin C (Ascorbic Acid)

The levels of ascorbic acid (Vitamin C) ranged from 19 mg/100g in honey from Tringalwadi of Igatpuri tehsil to 22 mg/100g in honey from Kasabe Sukene of Niphad tehsil. Vitamin C is an important antioxidant that supports immune function. The relatively high levels of Vitamin C in these samples suggest that honey can offer potential health benefits when consumed regularly. The slightly acidic nature of honey helps prevent the growth of spoilage organisms.

### 4. Amino Acids

Amino acids are essential for the nutritional value of honey. In this study, the amino acids proline and valine were measured.

- Proline levels ranged from 49 mg/100g in honey from Satana town of Satana tehsil to 57 mg/100g in honey, from Kasabe Sukene of Niphad tehsil showing the highest content. Higher proline levels are often associated with better-quality honey.
- Valine concentrations ranged from 4 mg/100g in honey from Wangan Sule of Surgana tehsil to 8 mg/100g in honey from Satana town of Satana tehsil. Valine is a branched-chain amino acid that contributes to honey's protein content. The variation in valine levels can be attributed to differences in nectar sources, as different flowers contribute distinct amino acid profiles to honey.

### 5. pH

The pH of honey is an important factor affecting its flavor, preservation, and antimicrobial properties. In this study, pH values ranged from 3.8 (Sample from Wangan Sule of Surgana tehsil) to 4.1 (Sample from Satana town of Satana tehsil), which is typical for honey.

### 6. Pesticide Residue Analysis

The presence of pesticide residues in honey is a serious concern for food safety. In this study, pesticide residues were detected in honey samples from Satana town of Satana tehsil and Kasabe Sukene of Niphad tehsil using gas chromatography-mass spectrometry (GC-MS), while no residues were found in Samples from Wangan Sule of Surgana tehsil and Tringalwadi of Igatpuri tehsil which are undisturbed wild areas. Pesticide contamination in honey typically arises from agricultural chemicals used near areas. The detection of pesticide residues in Samples from Satana village of Satana tehsil and Kasabe Sukene of Niphad tehsil highlights the potential impact of advanced agricultural practices on honey contamination. While pesticide levels in honey are regulated, the presence of these residues emphasizes the need for more stringent monitoring and enforcement of residue limits to ensure the safety and quality of honey for consumers.

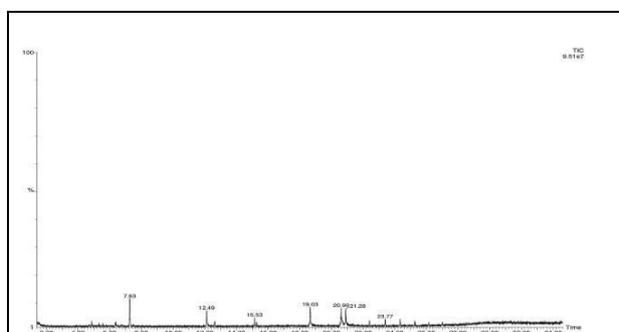


Figure 1 GCMS profile for sample 1(Wangan Sule, Surgana tehsil)

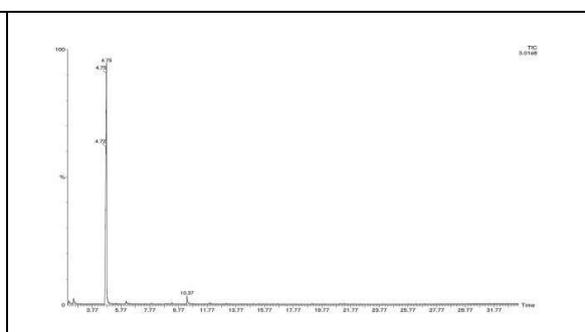


Figure 2 GCMS profile for sample 2 (Triangalwadi, Igatpuri tehsil)

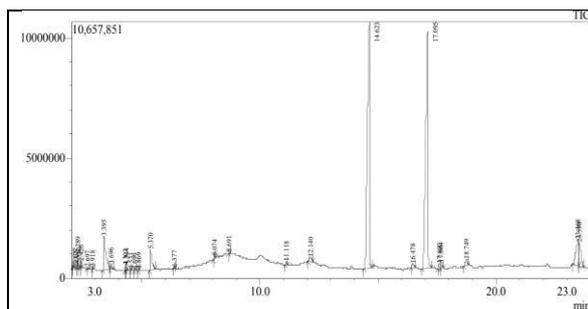


Figure 3 GCMS profile for sample 3 (Satana town, Satana tehsil)

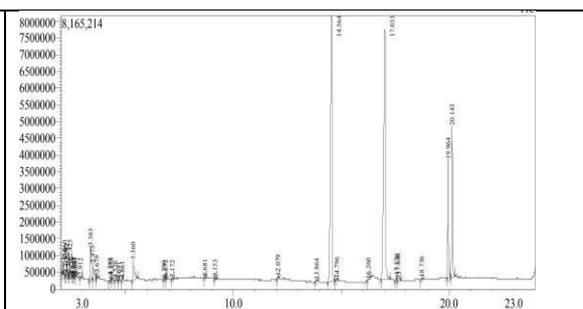


Figure 4 GCMS profile for sample 4 (Kasabe-Sukene, Niphad tehsil)

### Summary of Pesticide Residues Detected

In this study, several harmful pesticide-related compounds were identified in honey samples. These included organophosphates, phosphorodithioates, and other pesticide markers like phenthoate, profenofos, propiconazole, and o-butyl 0,0-diethyl phosphorothioate. The presence of these substances highlights the occurrence of pesticide residue in honey, which can be attributed to agricultural practices and the use of chemical treatments to protect crops during pollination. This contamination of honey with harmful chemicals rises significant concern about food safety and potential health risks to consumers.

#### 1. Phosphorodithioates and Organophosphates

The detected compounds, such as phosphorodithioic acid, 0,0,S-trimethyl ester, o-butyl 0,0-diethyl phosphorothioate, and phenthoate, belong to the class of organophosphates or phosphorodithioates, commonly used as insecticides. These chemicals function by inhibiting acetylcholinesterase, an enzyme essential for proper nerve function in insects, and potentially in humans.

#### 2. Azole Fungicides: Propiconazole

Another important compound found in the honey samples is propiconazole, a triazole fungicide widely used to control fungal diseases in crops. The continued presence of propiconazole in honey suggests that agricultural runoff and nearby pesticide applications may contribute to its accumulation in honey products.

Following table shows components detected in GC-MS profiling-

Sr. No.	Sample 1	Sample 2	Sample 3	Sample 4
1	Oct-(2E)-enyl-Butanoic acid ester	Allyl alcohol	Hexanoic acid, undecyl ester	Tricyclo[5.2.1.0(2,6)]dec-4-ene, 4-methyl-
2	Terpinen-4-ol	Furfural	Benzene, 1,2,3,4-tetramethyl-	2,4-Dimethylstyrene
3	3-N-hexylthiolane, S,S-dioxide	Phenol	Phosphorodithioic acid, O,O,S-trimethyl ester	Naphthalene, 1,2,3,4-tetrahydro-
4	n-Hexadecanoic acid	Guaiacol	Benzaldehyde, 2,4-dimethyl-	Phosphorodithioic acid, O,O,S-trimethyl ester
5	Chloromethyl 2-chloro undecanoate	4-hexanoyl resorcinol	Benzaldehyde, 4-ethyl-	Cyclopentanecarboxylic acid, 3-methylene-, methyl
6	Octadecanoic acid	Syringol	Benzeneacetic acid, ethyl ester	Naphthalene
7	2,2,11,11-Tetramethyl dodecane	1,2,3-Trimethoxy-5-methyl benzene	Phenol, 4-bromo-2-chloro-	Cyclopentanecarboxylic acid, 3-methylene-, methyl

8	2,2,4,4,6,8,8-heptamethyl nonane		beta.-d-Lyxofuranoside, heptyl-2,3-O-ethylboraned	Benzaldehyde, 2,5-dimethyl-
9	2,2,4,10,12,12-Hexamethyl-7-(3,5,5-trimethyl hexyl) tridecane		o-Butyl O,O-diethyl phosphorothioate	Benzene, 2,4-dimethyl-1-(1-methylpropyl)-
10			Propanetrione, diphenyl-	Benzaldehyde, 2,4-dimethyl-
11			Propanedioic acid, mononitrile, monohydrazide, N2	Benzeneacetic acid, ethyl ester
12			Naphthalene, 1,6-dimethyl-	Phenol, 4-bromo-2-chloro-
13			Naphthalene, 2,3-dimethyl-	Naphthalene, 1-methyl-
14			Ethyl (phenylthio)acetate	4(1H)-Pyrimidinone, 2-(ethylthio)-
15			o-Butyl O,O-diethyl phosphorothioate	o-Butyl O,O-diethyl phosphorothioate
16			9-Octadecen-1-ol, (E)-	Propanetrione, diphenyl-
17			Tricyclo[3.3.1.1(3,7)]decane-2,6-dione, 4-bromo-	Dodecane, 2,5-dimethyl-
18			Hexadecanoic acid, methyl ester	Naphthalene, 1,6-dimethyl-
19			9-Octadecenal, (Z)-	Naphthalene, 2,3-dimethyl-
20			Phenthoate	Ethyl (phenylthio)acetate
21			Oleic Acid	1-Hexadecanol
22			Profenofos	Decane, 5-propyl-
23			Tetracosanoic acid, methyl ester	Benzene, 1-methyl-2-[(3-methylphenyl)methyl]-
24			Cholesta-3,5-diene	Benzyltriethoxysilane
25			Cholest-5-en-3-ol (3.beta.-), nonanoate	Heptadecane, 8-methyl-
26				n-Hexadecanoic acid
27				Profenofos
28				Phenthoate
29				anti-10-Methyl-endo-tricyclo[5.2.1.0(2.6)]decane
30				Naphthalene, 2,3-dimethyl-
31				Ethyl (phenylthio)acetate
32				Propiconazole

Table 3- TIC after GCMC profiling of honey samples

## DISCUSSION-

Variation in sugar levels, amino acids, enzymes and Vitamin C (ascorbic acid) observed in honey samples indicates variation in floral origin. The GSO standard specifies that fructose should make up 27-44.3% of the total composition in honey, glucose should comprise 22-40.7% and no more than 5% sucrose in honey (Alghamdi, B. A. et al.2020). Glucose content influences the crystallization rate of honey, with higher glucose levels typically leading to faster crystallization (Khalil et al., 2012). In this study, estimated samples show fructose level ranges between 37-40.38%, glucose level ranges between 30-33.93%, sucrose level is between 1.35-2.46% as per GSO standards. The GSO standard requires the fructose/glucose ratio to be not less than 1. Estimated samples show fructose/glucose ratio more than 1. This means that, in high-quality honey, there should be at least as much fructose as glucose. This balance is important because it affects the texture, sweetness, and crystallization of honey. A ratio lower than 1 might indicate honey adulteration or an unnatural composition. The variation in amino acids levels can be attributed to differences in nectar sources, as different flowers contribute distinct amino acid profiles to honey (Isidorov et al., 2020). The GSO standard specifies that honey's pH should fall within the range of 3.24

to 6.1. The pH of estimated samples ranges between the limits of GSO standards. The pH on acidic side prevents microbial growth. The pH value also contributes to the flavour and stability of honey.

Pesticide residues are detected in the honey samples from highly advanced agricultural region. The persistence of Phosphorodithioates and Organophosphates in the environment, including in honey, raises concerns about chronic exposure, especially due to their neurotoxic effects (Sun et al., 2019). Phenthoate is of particular concern because it is a broad-spectrum insecticide that can have prolonged effects on non-target organisms, including honeybees, as well as humans if consumed in significant amounts (Méndez et al., 2021). Profenos, another organophosphate, is significant due to its link to harmful health effects, including toxicity to the respiratory and nervous systems. Research indicates that even low-level exposure to profenos can negatively impact human health (Tosi et al., 2020). The detection of propiconazole is concerning, as triazoles are known to interfere with hormonal systems in both humans and animals by inhibiting the production of steroid hormones (Zhou et al., 2021). Prolonged exposure to propiconazole has been associated with endocrine disruption, liver toxicity, potential carcinogenic effects (Zhou et al., 2021). These pesticide residues present health risks, particularly to children and pregnant women, as they can accumulate over time, leading to neurotoxic, endocrine disrupting, or carcinogenic consequences (Guillette et al., 2020).

### **Health Risks to Humans**

Pesticides have been found in honey samples from areas with agricultural practices. The presence of pesticide residues in honey can pose various health risks to humans. Although the pesticide levels in samples were below the regulatory thresholds set by organisations such as European food safety authority (EFSA) and the U.S. Environmental Protection Agency (EPA), long-term accumulation of these residues through regular consumption could have adverse effects. Continuous exposure to low levels of neurotoxic substances like organophosphates and fungicides such as propiconazole may contribute to developmental disorders, immune suppression, and an increased risk of cancer (Guillette et al., 2020).

Honey, being a widely consumed food-especially in traditional and alternative medicine-may be ingested in larger quantities, amplifying the risks of even low concentrations of pesticide residues. This is particularly concerning for vulnerable populations, including children, pregnant women, and those with weakened immune systems (Tosi et al., 2020).

### **Implications for Honey Production and Food Safety**

The detection of pesticide residues in honey emphasizes the need for stringent monitoring of agricultural practices, particularly pesticide use in areas where honey is produced. Honeybees, which are particularly vulnerable to pesticide exposure, can carry tainted pollen and nectar into the honey they create, thereby contributing to pesticide contamination. This underscores the need for stricter enforcement of maximum residue limits (MRLs) to ensure that honey complies with safety regulations and does not pose health dangers to consumers. Typically, the Maximum residue limits (MRLs) for the pesticides in honey are set at 0.05mg/kg (or 0.05 ppm), based on the limit of quantification. Nevertheless, some pesticides may have specific MRLs that vary depending on their intended use (EFSA, 2022).

Implementing certified organic farming methods has the potential to decrease the levels of pesticide residue found in honey, since organic regulations limit or ban the application of synthetic pesticides. Promoting organic beekeeping practices may enhance honey quality and lessen the chances of harmful pesticide contamination.

### **Environmental Impact of Pesticide Residues**

The environmental consequences of pesticide residues in honey extend beyond human health. Persistent pesticide contamination can pollute soil and water systems, negatively affecting not only honeybees but also other pollinators and wildlife. Given the critical role that bees play in pollination and maintaining biodiversity, the widespread use of harmful pesticides jeopardizes ecological balance and agricultural sustainability (van der Sluijs et al., 2020). Consequently, addressing pesticide contamination in honey should be part of broader efforts to regulate pesticide use and safeguard pollinator health.

### **Conclusion and future direction-**

The biochemical analysis of honey samples revealed variations in sugar composition, enzyme activity, vitamin content, amino acid concentrations, and pH. These differences reflect the diverse sources and environmental conditions under which the honey was produced. However, the detection of pesticide residues in two of the samples raises concerns about contamination from nearby agricultural activities. While the sugar and nutrient levels in the samples met expected standards, the presence of pesticides underscores the importance of improving regulatory oversight and monitoring to protect the safety and

quality of honey. The identification of harmful pesticide residues, such as phenthoate, profenofos, and propiconazole, in honey samples raises serious concerns about food safety and public health. In this research, it is observed that honey from undisturbed areas (without anthropologic activities) is pure and suitable to consume but honey from civilized and agriculture areas is contaminated. Honey from wild areas where pesticides are not introduced can be consumable. But the honey from highly advanced agricultural areas might be contaminated with pesticide residue. It should be avoided. Although the detected levels were within current regulatory limits, chronic exposure to these harmful substances could lead to significant health issues. It is crucial to enhance monitoring, adopt safer agricultural practices, and promote organic farming to minimize pesticide contamination in honey. These findings highlight the need for continued research on the sources, prevalence, and health impacts of pesticide residues in honey and other food products.

This study provides valuable insights into the chemical composition and pesticide contamination levels in honey, emphasizing the importance of addressing the issue of pesticide residues. Honey continues to be a popular natural sweetener with the various health benefits, but the potential presence of pesticide is becoming a growing concern that needs more research. Future studies should explore the long-term impact of pesticide exposure from honey, enhance farming practices, and create stricter regulations to ensure honey production remains safe and sustainable.

#### REFERENCES-

1. Afrin, S., Haneefa, S. M., Fernández-Cabezudo, M. J., Giamperi, F., al-Ramadi, B. K., & Battino, M. (2020). Therapeutic and preventive properties of honey and its bioactive compounds in cancer: An evidence-based review. *Nutrition Research Reviews*, 33(1), 50-76. <https://doi.org/10.1017/S0954422419000192>
2. Alghamdi, B. A., Alshumrani, E. S., Saeed, M. S. B., Rawas, G. M., Alharthi, N. T., Baeshen, M. N., Helmi, N. M., Alam, M. Z., & Suhail, M. (2020). Analysis of sugar composition and pesticides using HPLC and GC-MS techniques in honey samples collected from Saudi Arabian markets. *Saudi Journal of Biological Sciences*, 27(12), 3720–3726. <https://doi.org/10.1016/j.sjbs.2020.08.018>
3. Alvarez-Suarez, J. M., Giampieri, F., González-Paramás, A. M., & Bompadre, S. (2013). Honey as a source of bioactive compounds: Health benefits and medicinal properties. *Journal of Agricultural and Food Chemistry*, 61(50), 12134–12150. <https://doi.org/10.1021/jf402110m>
4. American Association of Cereal Chemists (AACC). (2000). *Approved methods of the AACC* (10th ed.).
5. AOAC International. (1990). *Official methods of analysis of AOAC International* (15th ed., Method 967.21: Ascorbic acid (vitamin C) in foods, 2,6-dichlorophenolindophenol titrimetric method).
6. Baroni, M. V., Arrua, C., Nores, M. L., Faye, P., Diaz, M. D. P., & Chiabrando, G. A. (2009). Composition of honey from Cordoba (Argentina): Assessment of North/South provenance by chemometrics. *Food Chemistry*, 114, 727–733. <https://doi.org/10.1016/j.foodchem.2008.10.004>
7. Bergmeyer, H. U., & Bernt, E. (1974). *Methods of enzymatic analysis*. In *Methods of enzymatic analysis* (Vol. 2, pp. 1-12). Academic Press.
8. Bilandzic, N., Dokic, M., Sedak, M., Kolanovic, B. S., Varenina, I., Koncurat, A., & others. (2011). Determination of trace elements in Croatian floral honey originating from different regions. *Food Chemistry*, 128(4), 1160–1164. <https://doi.org/10.1016/j.foodchem.2011.04.080>
9. Blasa, M., Candiracci, M., Accorsi, A., Piacentini, M. P., Albertini, M. C., & Piatti, E. (2006). Raw Millefiori honey is packed full of antioxidants. *Food Chemistry*, 97(2), 217–222. <https://doi.org/10.1016/j.foodchem.2005.04.031>
10. Boesel, P. A., & Galgano, F. L. (1987). Determination of sucrose content in juices by difference. *Journal of Agricultural and Food Chemistry*, 35(4), 669–671. <https://doi.org/10.1021/jf00082a026>
11. Bogdanov, S. (1999). *Harmonised methods of the International Honey Commission: IHC responsible for the methods*. Swiss Bee Research Centre, FAM. Liebefeld, Switzerland. Available from: [http://www.usamvcluj.ro/Diagnoza/IHCmethods\\_e.pdf](http://www.usamvcluj.ro/Diagnoza/IHCmethods_e.pdf)
12. Bogdanov, S. (2010). [Nutritional and functional properties of honey]. *Voprosy Pitaniia*, 79(6), 4–13. PMID: 21395098
13. Bogdanov, S., & Oddo, L. P. (2004). Determination of honey botanical origin: Problems and issues. *Apidologie*, 35(S2–S3). <https://doi.org/10.1051/apido:2004047>
14. Bontempo, P., Karagiannis, S., & Tsigouri, A. (2018). Honey production and consumption in Greece. *Journal of Apiculture Research*, 57(4), 451–459. <https://doi.org/10.1080/00218839.2018.1491542>
15. Crane, E. (1979). *Honey: A comprehensive survey*. Heinemann, International Bee Research Association (IBRA).
16. Cunningham, M. M., Tran, L., McKee, C. G., Ortega Polo, R., Newman, T., Lansing, L., Griffiths, J. S., Bilodeau, G. J., Rott, M., & Guarna, M. M. (2022). Honey bees as biomonitors of environmental contaminants, pathogens, and climate change. *Ecological Indicators*, 134, 108457. <https://doi.org/10.1016/j.ecolind.2021.108457>
17. da Silva, P. M., Gauche, C., Gonzaga, L. V., & Costa, A. C. O. (2016). Honey: Chemical composition, stability, and authenticity. *Food Chemistry*, 196, 309–323. <https://doi.org/10.1016/j.foodchem.2015.09.051>
18. European Food Safety Authority (EFSA). (2022). Modification of the existing maximum residue levels for acetamiprid in honey and various oilseed crops. *EFSA Journal*, 20(7), 7535. <https://doi.org/10.2903/j.efsa.2022.7535>
19. FAO (Food and Agriculture Organization). (2021). *Honey and bee products: Global trends*.
20. French Beekeepers' Association (Fédération Française des Apiculteurs). (2021). *Honey consumption in France*.

21. Gao, J., Li, X., & Zhang, H. (2019). Honey consumption in traditional Chinese medicine and its health benefits. *Journal of Ethnopharmacology*, 245, 112146. <https://doi.org/10.1016/j.jep.2019.112146>
22. German Honey Association. (2019). Honey consumption and its role in German nutrition.
23. Gismondi, A., Di Marco, G., & Canini, A. (2017). Detection of plant microRNAs in honey. *PLoS ONE*, 12(3), e0172981. <https://doi.org/10.1371/journal.pone.0172981>
24. Guillette, E. A., Acosta, D., & Torres, J. (2020). Pesticide exposure and endocrine disruption in honey: Health risks to consumers. *Environmental Health Perspectives*, 128(9), 96001. <https://doi.org/10.1289/EHP693>
25. Guillette, E. A., Acosta, D., & Torres, J. (2020). Pesticides and endocrine disruption: A review of the evidence. *Environmental Health Perspectives*, 128(5), 057004. <https://doi.org/10.1289/EHP6671>
26. Guler, A., Bakan, A., Nisbet, C., & Yavuz, O. (2007). Determination of important biochemical properties of honey to discriminate pure and adulterated honey with sucrose (*Saccharum officinarum* L.) syrup. *Food Chemistry*, 105, 1119–1125. <https://doi.org/10.1016/j.foodchem.2007.04.011>
27. <https://pubmed.ncbi.nlm.nih.gov/articles/PMC6218329/#:~:text=2.,2.>
28. Isidorov, V. A., Stafie, C., & Szefer, P. (2020). Amino acid composition of honey from different floral origins. *Food Chemistry*, 319, 126546. <https://doi.org/10.1016/j.foodchem.2020.126546>
29. Italian Ministry of Agriculture. (2019). Honey consumption in Italy and its role in traditional cuisine.
30. Japanese Honey Industry Report. (2020).
31. Juszczak, L., Socha, R., Roznowski, J., Fortuna, T., & Nalepka, K. (2009). Physicochemical properties and quality parameters of herb honeys. *Food Chemistry*, 113(2), 538–542. <https://doi.org/10.1016/j.foodchem.2008.08.074>
32. Khalil, M. I., Sulaiman, S. A., & Gan, S. H. (2012). Honey composition and properties. In *Honey in Traditional and Modern Medicine* (pp. 69-85). Springer. [https://doi.org/10.1007/978-1-4614-6435-8\\_5](https://doi.org/10.1007/978-1-4614-6435-8_5)
33. Layne, E. A. (1957). Spectrophotometric and calorimetric methods for the determination of reducing sugars. *Journal of Biological Chemistry*, 228(1), 425–432.
34. Méndez, J., Pérez, M., & Rodríguez, A. (2021). Pesticide residues in honey: Occurrence, analytical methods, and toxicological considerations. *Environmental Toxicology and Chemistry*, 40(3), 863-876. <https://doi.org/10.1002/etc.5062>
35. Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, 31(3), 426–428. <https://doi.org/10.1021/ac60147a023>
36. Miller, J. M. (2002). *Mass spectrometry: A textbook*. Springer.
37. Montgomery, R. (1957). Determination of sugar with the resorcinol reagent. *Analytical Chemistry*, 29(3), 501–505. <https://doi.org/10.1021/ac60123a009>
38. Naik, R. S., Sharma, P., & Verma, A. (2015). Honey as a therapeutic agent: Its uses in traditional medicine. *International Journal of Green Pharmacy*, 9(4), 244-250.
39. Nanda, V., Singh, B., Kukreja, V. K., & Bawa, A. S. (2009). Characterization of honey produced from different fruit plants of northern India. *International Journal of Food Science & Technology*, 44(12), 2629–2636. <https://doi.org/10.1111/j.1365-2621.2009.02180.x>
40. National Honey Board. (2020). Honey consumption in the U.S.
41. National Library of Medicine. (n.d.). PubChem compound database. U.S. National Library of Medicine. <https://pubchem.ncbi.nlm.nih.gov/>
42. Oddo, L. P., & Bogdanov, S. (2004). Determination of honey botanical origin: Problems and issues. *Apidologie*, 35(S2), S2–S3. <https://doi.org/10.1051/apido:2004053>
43. Perkin Elmer Clarus 600C GC and MS System Manual. (n.d.). For system-specific information, configuration, and maintenance guidelines. Perkin Elmer Website.
44. Pita-Calvo, C., Guerra-Rodríguez, M. E., & Vázquez, M. (2017). Analytical methods used in the quality control of honey. *Journal of Agricultural and Food Chemistry*, 65(3), 690-703. <https://doi.org/10.1021/acs.jafc.6b04776>
45. Puścion-Jakubik, A., Borawska, M. H., & Socha, K. (2020). Modern methods for assessing the quality of bee honey and botanical origin identification. *Foods*, 9(8), 1028. <https://doi.org/10.3390/foods9081028>
46. Rahman, M. M., Alam, M. N., Fatima, N., Shahjalal, H. M., Gan, S. H., & Khalil, M. I. (2017). Chemical composition and biological properties of aromatic compounds in honey: An overview. *Journal of Food Biochemistry*, 41(6), e12405. <https://doi.org/10.1111/jfbc.12405>
47. Randox Laboratories Ltd. (2024). Glucose Oxidase Reagent. Available from: <https://www.randox.com>
48. Sadeghi, N., & Amini, M. (2010). Amylase activity measurement using dinitrosalicylic acid reagent. *International Journal of Biological Macromolecules*, 46(2), 182–185. <https://doi.org/10.1016/j.ijbiomac.2009.12.010>
49. Sadeghi, N., & Amini, M. (2010). Amylase activity measurement using dinitrosalicylic acid reagent. *International Journal of Biological Macromolecules*, 46(2), 182–185. <https://doi.org/10.1016/j.ijbiomac.2009.11.003>
50. Sahin, S., & Karadavut, U. (2018). The importance of honey in Turkish culture and health benefits. *Journal of Apiculture*.
51. Silva, L. R., Videira, R., Monteiro, A. P., Valentão, P., & Andrade, P. B. (2009). Honey from Luso region (Portugal): Physicochemical characteristics and mineral contents. *Microchemical Journal*, 93(1), 73–77. <https://doi.org/10.1016/j.microc.2009.01.004>
52. Singh, R. (2021). Current honey market in India - Volume and value. *International Journal of Ayurveda and Pharma Research*, 9(8), 82-88. <https://doi.org/10.47070/ijapr.v9i8.2050>
53. Smith, K. E., Weis, D., Scott, S. R., Berg, C. J., Segal, Y., & Claeys, P. (2021). Regional and global perspectives of honey as a record of lead in the environment. *Environmental Research*, 195, 110800. <https://doi.org/10.1016/j.envres.2021.110800>
54. Somogyi, M. (1952). Notes on sugar determination. *Journal of Biological Chemistry*, 195(1), 19–23.
55. Sun, L., Liu, X., Zhang, Y., & Li, J. (2019). Analysis of pesticide residues in honey using advanced analytical techniques. *Environmental Science and Pollution Research*, 26(4), 3884-3894. <https://doi.org/10.1007/s11356-019-04655-w>
56. Tosi, E. A., Moretti, M., & Mancini, M. (2020). Safety assessment of pesticide residues in honey: A global review. *Food Control*, 112, 107113. <https://doi.org/10.1016/j.foodcont.2020.107113>

57. Trifković, J., Andrić, F., Ristovojević, P., Guzelmeric, E., & Yesilada, E. (2017). Analytical methods in tracing honey authenticity. *Journal of AOAC International*, 100(4), 827-839. <https://doi.org/10.5740/jaoacint.17-0142>
58. U.S. Department of Agriculture (USDA). (2021). Report on honey production.
59. van der Sluijs, J. P., Pochi, D., van der Kroon, P., & Jansen, R. (2020). The effects of pesticide use on honeybees and other pollinators. *Science of The Total Environment*, 726, 138567. <https://doi.org/10.1016/j.scitotenv.2020.138567>
60. White Jr, J. W. (1978). Honey. In C. O. Chichester, E. M. Mrak, & G. F. Stewart (Eds.), *Advances in Food Research* (pp. 288–374). Academic Press.
61. Wolfe, R. J., & Somogyi, M. (1944). A new method for the quantitative determination of maltose in sugar solutions. *Journal of Biological Chemistry*, 151(1), 275–285.
62. World Honey Market Trends. (2020). China's role in global honey production and consumption.
63. Zhao, C., & Shuhua, Z. (2011). Determination of fructose in honey using resorcinol reagent method. *International Journal of Food Science & Technology*, 46(2), 423–429. <https://doi.org/10.1111/j.1365-2621.2010.02561.x>
64. Zhao, L., Zhang, X., Li, C., & Liu, X. (2020). Sugar profiles and sweetness index of honey from different floral sources. *Food Control*, 111, 107079. <https://doi.org/10.1016/j.foodcont.2020.107079>
65. Zhou, R., Li, Q., Zhang, Z., & Wang, J. (2021). Propiconazole and its effects on endocrine disruption and human health. *Environmental Toxicology and Chemistry*, 40(2), 413-421. <https://doi.org/10.1002/etc.5047>
66. Zhu, Y., & Wang, H. (2011). Invertase activity and honey quality. *Journal of Food Science*, 76(1), C118–C123. <https://doi.org/10.1111/j.1750-3841.2010.02031.x>