

# Pesticide Footprints in Fenugreek: Residue Quantification in Market-Sourced Fresh Green Seeds from the Udaipur-Chittorgarh Belt

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## Abstract

As has been true throughout human history, India is the world's biggest producer of fenugreek and its main consumer for both culinary and medicinal uses. The leaves and seeds of fenugreek, a significant leguminous annual herb, are utilized throughout North Africa and India. Fenugreek seeds serve purposes as a flavoring element, as a condiment, and in larger amounts in pan cakes and soups. The current study intends to identify the various pesticidal residues in *Trigonella foenum-graecum* seeds purchased from several marketplaces in the Udaipur-Chittorgarh belt since pesticides offer serious health risks and envisage the possible health repercussions. Results revealed the residues of 13 different pesticides in the fresh green seeds of *Trigonella foenum-graecum* obtained from markets of Dabok, Mangalwar, Kapasan, Mavli and Fatehnagar of Udaipur-Chittorgarh belt. Many of the pesticides found in samples are not approved by the Central Insecticide Board and Registration Committee (CIBRC) indicating an off-label use of pesticides. The health risk assessment indicated that the percentage contribution to the Acceptable Daily Intake (ADI) of pesticides from dietary intake of fenugreek varied between 29.7% and 133% for children and between 12.6% and 74.2% for adults. Although the Health Risk Index (HRI) values were below 1, the analysis showed that children face a higher risk due to the levels of malathion found in the samples. Therefore, the current findings underscore the necessity for careful monitoring of pesticide levels and conducting health risk assessments.

**Keywords:** *Trigonella foenum-graecum*, Pesticide residues, Health risks, Maximum Residue Limit (MRL), Persistent organic pollutants.

## Introduction

Agriculture is a key pillar of the Indian economy. Currently, India holds the second position globally in vegetable production. Various types of vegetables are cultivated in India, categorized into different groups such as solanaceous, cucurbitaceous, leguminaceous, cruciferous, root vegetables and leafy greens across diverse regions. Leafy vegetables constitute the largest portion (53%) of the various types of vegetables, while root crops and fruit vegetables comprise 17% and 15%, respectively (Najmi et al. 2023). These vegetables are also very perishable and are often harvested multiple times. Common leafy vegetables such as spinach, onion greens and amaranth are low-value crops, which historically resulted in minimal pesticide use on them. However, in recent years, there has been an uptick in consumer demand and pest problems, leading to some pesticide application on these crops. The occurrence of pesticide residues is more prevalent in leafy vegetables since these crops are harvested frequently and are often consumed fresh or raw.

Additionally, the surface area of these crops is larger, so the initial application of pesticide spray is greater (Swaroop and Tamchos, 2024).

Approximately 4.6 million tonnes of chemical pesticides are applied worldwide each year, comprising more than 500 distinct varieties. Around 85% of these pesticides are employed on agricultural crops, while the remaining 15% are used for other applications. Although chemical pesticides have decreased crop losses due to pests, their extensive use has resulted in several adverse effects on the ecosystem. The toxicity of pesticides does not affect only the intended target species, it can also impact non-target organisms in the surrounding environment. The use of pesticides in agriculture poses significant environmental and health threats, as it leads to the accumulation of pesticide residues in crops and disrupts the biochemical processes of plants (Dar and Kaushik, 2023). Pesticides influence photosynthesis, the development of plant cells, respiration, biosynthetic processes, and molecular structure (Sohrabi et al. 2024). In recent times, there has been extensive debate regarding the risks associated with the use of chemical pesticides. A considerable quantity of pesticide residues is attributed to spraying and seed treatments. To minimize pesticide residue levels on fresh produce, pre-harvest intervals (PHI) or safe waiting times have been suggested for vegetables. Moreover, for each pesticide, Maximum Residue Limits (MRLs) are established for every crop, during which time the pesticide levels in the crop fall below the MRL thresholds. The Pre Harvest Interval (PHI) or Waiting Period can be established by performing field trials on crops cultivated using sound agricultural practices. It is the duration between the final application and the initial gathering of the crops. PHI is specific to each crop and pesticide, meaning that each pesticide used on each crop has a distinct value (Yami et al. 2025). Due to significant market demand and a lack of awareness regarding the harmful pesticide impacts that could affect human health after consuming these contaminated vegetables. Therefore, evaluating the presence of pesticides in frequently eaten foods is essential for ensuring consumer safety.

Pesticides, insecticides, herbicides, and fungicides are man-made substances used in agriculture. Several nations prohibited the use of organochlorine pesticides due to their toxicological characteristics and harmful impacts. Nonetheless, certain nations persisted in utilizing these pesticides within the agricultural sector (Anaduaka et al. 2023). Worldwide, 50% of pesticides, 22% of insecticides, and 15% of fungicides were used (Maino et al. 2023).

The organophosphate pesticides most frequently used in the agricultural industry include monocrotophos, methyl parathion, diazinon, dichlorvos, chlorpyrifos, glyphosate, trichlorfon, and malathion (Mali et al. 2023). Monocrotophos (MCP) is an organophosphate pesticide widely used in agricultural crops such as rice, maize, cotton, sugarcane, soybeans, groundnuts, and various vegetables. MCP dissolves easily in water, leading to decreased sorption in soil. This results in MCP oozing into the groundwater, presenting a considerable risk of contamination. The half-life of the MCP is influenced by temperature and pH level, estimated to be between 17 and 96 days. However, the half-life of technical grade MCP can potentially exceed 2500 days if it is stored correctly at 38 °C in a polyethylene container or glass under stable conditions. It leads to irregularities, varying from slight to intense confusion, restlessness, excessive saliva production, seizures, respiratory failure and aging in insects and mammals. MCP impacts humans by

blocking the function of the acetylcholine esterase enzyme. MCP is responsible for the catalytic breakdown of acetylcholine and influences the communication between neurons (Rajan et al. 2024).

Malathion is a versatile organophosphate insecticide utilized to manage numerous insects in farming, public health and for the treatment of head lice in people. It's a contact-acting, non-systemic insecticide and acaricide that is utilized in mosquito control initiatives and for managing household pests. Malathion (O,O-dimethyl-S-1,2-bis ethoxy carbonyl ethyl phosphorodithionate) is widely utilized globally. Fewer than 1% of Malathion applications affect pests, as most of it is released into the environment, potentially contaminating soil and water sources. Malathion might pose potential adverse effects on human health because: (1) it possesses a lipophilic characteristic that could reduce its solubility in water; (2) it has a significant bioaccumulation rate in the ecosystem; and (3) its physical and chemical traits enhance its persistence in the environment (Yadav et al. 2025). The harmful health impacts of Malathion result from the suppression of the enzyme known as acetylcholinesterase. The impacts on internal organs like the heart and liver, along with genetic and chromosomal conditions, have been recorded as well (Li et al. 2024).

Dimethoate, an organophosphate pesticide, was first created in 1962 for comprehensive control of various insects including mites, aphids, flies, and plant hoppers. It inhibits the activity of acetylcholinesterase, leading to nerve injury and the organism's demise. Numerous organisms have been noted to suffer damage from dimethoate. Among them, aquatic creatures are significantly impacted by their exposure to the pesticide through runoff. Consequently, it may exhibit oxidative stress, neurotoxic effects, and behavioral abnormalities. Numerous aquatic creatures are key components of the human diet. Consequently, the built-up pesticide residues in these organisms make their way into the human body. It could also enter the human body via groundwater (used for drinking) where residues were found because of leaching (Sadhu et al. 2024).

Chlorpyrifos [O,O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate] is an organophosphate pesticide, acaricide, and nematocide employed to manage a wide range of insect pests found in foliage and soil on various food and feed crops. It is regarded as one of the most widely utilized insecticides globally. Chlorpyrifos exhibits moderate toxicity to mammalian animals; nevertheless, because it inhibits the enzyme cholinesterase, it can be very dangerous in certain situations (Hussain et al. 2023). Because of its lipophilic nature, CPF can permeate the epidermis and infiltrate the animal's body, reaching tissues, organs, milk, and various bodily fluids. Furthermore, CPF residues have been found in fruits, grains, and vegetables. The detection of CPF residues in soils, natural waters, food, and humans and animals is not a recent issue; however, investigations into the prevalence and biodegradation of this pesticide across various environmental components and its effects on the ecosystem continue to be the focus of numerous researchers today (Wolejko et al. 2022).

Dichlorvos (2,2-Dichlorovinyl dimethyl phosphate, [DDVP]) is part of the organophosphate group and is commonly used as an insecticide in agricultural practices and storage facilities after harvest. Contact with DDVP can be harmful via swallowing, breathing, and skin absorption. Due to its solubility in both water and fat, DDVP can readily cross biological barriers and build up within the cytoplasm (Salem et al. 2023).

Therefore, the use of this pesticide can lead to different negative impacts on the ecosystem, helpful pests, and insects. It can affect the nervous systems of helpful insects, resulting in paralysis and eventual demise (Saravankumar et al. 2024).

Aldrin was initially presented as an agricultural pesticide, but its application was banned in numerous countries, including India, due to the Stockholm Convention on Persistent Organic Pollutants. Even with the ban in place, aldrin's lasting presence in the environment still creates significant problems. Aldrin's remnants are still present in various locations around the globe. The bioaccumulation of aldrin has taken place due to leftover pollution in soil and water, affecting wildlife and possibly endangering human health via exposure through the food chain (Kumari and Akhtar, 2024). Aldrin is a white crystalline substance utilized as a versatile soil pesticide for safeguarding numerous food crops, treating seeds, and controlling pest infestations including termites and ants. Aldrin is rapidly converted to dieldrin either in environmental settings or upon entering the human body. Aldrin toxicity is marked by heightened liver activity, headaches, nausea, dizziness, and general discomfort, along with vomiting, which subsequently leads to muscle spasms and convulsions (Kumar et al. 2025).

Phosalone is an insecticide and is part of the organic phosphorus toxin family. Phosalone is utilized to manage pests on fruit trees like apples, pears, grapes, cherries, sour cherries and peaches. This substance exhibits acute toxicity upon oral exposure and is gradually hydrolyzed in the liver. Phosalone is transformed into Phosaloneexon by environmental influences like oxygen and light, along with liver microsomal enzymes, resulting in increased toxicity (Armak et al. 2024).

Fipronil (FIP) is an effective and wide-ranging insecticide utilized to combat various arthropod pests, including parasitic mites and insect nuisances that impact both animals and plants. Due to its numerous advantages, FIP is extensively utilized in agriculture and veterinary medicine; however, its non-selective application may lead to ecotoxic consequences for non-target organisms. Fipronil (FIP) (5-amino-1-[2,6-dichloro-4-(trifluorometil) fenil]-4-[(trifluorometil)sulfinil]-1H-pirazol-3-carbonitrila), a chemical that originates from the phenylpyrazole class, is a second generation, very effective, systemic, and broad spectrum insecticide that is notable among the most utilized worldwide (Shahid et al. 2024). Regarding its mechanism of action and toxicity, FIP functions as an antagonist to the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) by attaching to ionotropic channels controlled by GABA types A and C (GABAA,c), thus blocking chloride channels in the central nervous system of insects. This obstruction process interferes with typical neural activities and, as a result, causes hyperexcitability, paralysis, and ultimately, the death of insects (Mamboungou et al. 2024).

Research shows that endosulfan, utilized as an insecticide, poses risks to human health and a diverse range of aquatic and terrestrial organisms. Featuring two isomeric forms referred to as  $\alpha$  and  $\beta$ -endosulfan, this compound exhibits a notable level of persistence across various environmental compartments, including the atmosphere, sediments, and water, as well as other significant compartments. Endosulfan is widely recognized for its harmful effects on human health and has been demonstrated to lead to detrimental consequences for various aquatic and terrestrial species (Kumari and Marathe, 2024). Endosulfan is a

commonly utilized, broad-range organochlorine pesticide and acaricide recognized for its application in several vegetables, plants, fruits, grains, and cotton. It was initially utilized in the 1950s to manage insects like cotton bollworm (*Helicoverpa zea* or *H. armigera*). Metabolites of endosulfan have been detected and documented in humans, milk from livestock, as well as in fruits and vegetables (Mayea and Haba, 2025). Consuming contaminated food or drinks is a common method of being exposed to harmful pesticides. Endosulfan, a disruptive endocrine agent enters the human body via consumption, inhalation, or smoking of the substance, tainted tobacco, skin exposure to polluted soil, proximate hazardous waste sites, or unintentional leaks. The water-soluble metabolites of endosulfan, primarily including ES, diol and ether metabolites, accumulate in the fatty tissues of mammals based on their lipophilicity, whereas in women, in addition to fatty tissues, it may also be present in milk, placenta and navel string (Bhanot and Pant, 2025).

Phorate is an extremely toxic organophosphorus insecticide commonly utilized in the cultivation of fruits and vegetables cultivation. Exposure to excessive amounts of phorate can cause acute poisoning symptoms including headache, nausea, dizziness, and loss of appetite (Patel and Hundet, 2025). In certain severe instances, pulmonary edema, cerebral edema, coma, and respiratory failure have been documented. Prolonged, low-level exposure to phorate may lead to decreased blood cholinesterase activity and symptoms like muscle twitching and excessive sweating. Recent studies uncovered that phorate might induce hyperglycemia and decrease the diversity of intestinal microbiota in mice (Li et al. 2024).

The pesticide bifenthrin is a member of the pyrethroid family. In addition to some mite species, it works well against a variety of pests that consume foliage, such as beetles, flies, bugs, cicadas, moths and grasshoppers. By interfering with sodium channels and impairing neuronal activity, bifenthrin has an impact on insects' neurological systems (Kim et al. 2023). As lipophilic pyrethroid insecticides, bifenthrin readily attaches to soil particles, contributing to their persistence in soil, with average residuals lasting between 60 to 75 days and potentially up to eight months. Because of its enduring characteristics and extensive application, bifenthrin has been found in different ecosystems and land-dwelling animals. For example, in California, surface water and sediment have shown levels of bifenthrin reaching 3.79  $\mu\text{g L}^{-1}$  and 437  $\text{ng g}^{-1}$ , respectively (Sung et al. 2023).

The aerial application of the phosphorothioate insecticide parathion on crops in the late 20th century greatly boosted harvests but led to elevated occupational toxicity among handlers and agricultural laborers, along with instances of deliberate self-poisoning, resulting in its prohibition in numerous Western nations by the early 2000s. Due to the low solubility and volatility of parathion, most available formulations utilized organic solvents such as xylene to enhance the effectiveness of the aerosols and dusts. Parathion, classified as a WHO hazard class 1b insecticide, is among the most harmful OP pesticides ever approved by the EPA, having been prohibited in the EU in 2001 and in the USA in 2003. *In vitro*, parathion serves as a weak AChE inhibitor ( $k_i = \sim 0.3 \times 10^1 \text{ M}^{-1}\text{min}^{-1}$ ), whereas *in vivo* exposure leads to its conversion to paraoxon ( $k_i = \sim 10^6 \text{ M}^{-1}\text{min}^{-1}$ ) via P450 in the liver. This results in swift inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) in both the peripheral and central nervous systems as well as in circulating red blood cells (Zhang et al. 2025). Exposure to parathion can lead to toxicity through inhaling aerosols or mists, through skin or eye contact from machinery and clothing, orally from food residue, or by deliberately

ingesting from a storage container. Clinical signs consist of nausea, vomiting, abdominal cramps and diarrhea, along with confusion, blurred vision, sweating, muscle spasms, irregular heartbeat, seizures and fatality (Rosenberg et al. 2023).

Triazophos, known by its IUPAC designation (diethoxy-[(1-phenyl-1,2,4-triazol-3-yl)oxy]-sulfanylidene-lambda5-phosphane) and having the molecular formula  $C_{12}H_{16}N_3O_3PS$ , serves as a broad-spectrum organophosphate insecticide, acaricide, and nematicide for safeguarding agricultural crops, fruits, vegetables and oilseeds (Loganathan et al. 2024). Extensive use of triazophos resulted in residues in various crops, including 1.87 mg/kg found in wheat grains (Cang et al. 2024). It was additionally discovered that triazophos residues in apple, cabbage, rice and wheat flour exceeded an unacceptable level in China. Triazophos residues will enter aquatic ecosystems through surface runoff, direct overspray, or rainfall. Exposure to triazophos resulted in multiple toxic effects on aquatic life, including harm to the photosynthetic mechanism in *Chlorella pyrenoidosa* (Su et al. 2022), metabolic disruptions in female green mussels developmental issues in embryos and larvae of rare minnows (*Gobiocypris rarus*) and oxidative stress in zebrafish (Liu et al., 2023).

Current lifestyles, eating habits, mental stress, environmental influences and the extensive use of processed ingredients in food production and agriculture have all significantly accelerated the emergence of several health issues. Worldwide, scientists have been searching for natural pharmaceutical compounds that could be used to treat lifestyle-related illnesses or postpone their development. Fenugreek seeds have attracted considerable interest across multiple domains, such as health, nutrition and advanced nanotechnology uses, owing to their diverse properties (Faisal et al. 2024).

Fenugreek (*Trigonella foenum-graecum* L.), a yearly leguminous species from the Fabaceae family, is extensively used around the world. Hailing from the Indian subcontinent and the Eastern Mediterranean, fenugreek ranks among the oldest recognized medicinal plants and is appreciated in these areas as food, spice and traditional remedy (Aludatt et al. 2024). Fenugreek seeds are rich in nutrients and bioactive substances, and studies emphasize their numerous health advantages, such as anti-inflammatory, anti-cancer and antioxidant effects (Coban, 2025). Fenugreek seeds, leaves and extracts have shown encouraging effectiveness in controlling diabetes, as evidenced by rat models where diabetes induced by streptozotocin was treated with fenugreek seed extract. Germinated fenugreek seeds show greater antioxidant content and enhanced antidiabetic benefits than boiled seeds. This is due to the enhanced release or bioavailability of bound antioxidants when germination occurs. The differences in antioxidant properties are affected by environmental factors, genotype and the chemical composition of seeds (Haxhiraj et al. 2024).

Thus, the objective of this research is to determine the multi-residues of different pesticides in the fresh green seeds of a significant annual herb, Fenugreek. Given the significant health risks associated with pesticides, the current research also determines the levels of pesticide exposure and forecasts the possible health effects of different pesticide residues on fenugreek.

## Maximum Residue Limits

Maximum residue limits (MRLs) refer to the highest concentration of pesticide residues (measured in mg of residue per kg of food or animal feed, mg/kg) that may be found in or on food due to pesticide application in accordance with good agricultural practice (GAP) and product labeling directions. The CODEX Alimentarius Commission of FAO/WHO sets MRLs for different pesticides globally. In the 1990s, a problem arose with harmonizing MRLs according to CODEX because of a lack of an international standard and importers' acceptance of the exporter's MRL. Since then, numerous countries have been setting up nationally based MRLs. MRL values are also required to determine the waiting periods or pre-harvest intervals for the crops. Each pesticide has varying MRL values depending on the crop. When a pesticide is approved for a specific crop, the MRL for that pesticide in the crop is typically established based on values derived from monitored field residue studies. Nonetheless, if a pesticide lacks approval for application on a crop, the MRL may be established at the limit of determination (LoD). In the EU, the standard minimum LoD is 0.01 mg/kg. To establish the MRL of a pesticide for a particular commodity, two types of trials must be conducted. Numerous research projects concerning MRL levels have been carried out over the past few decades.

**Table: 1 Maximum Residue Limit of the Pesticides found in Studied Samples as Per CODEX Alimentarius Commission**

| S.no.                                                                                                                                                                                                                                                                                                                                                                                                   | Pesticide             | MRL        | Commodity                  | Year of Adoption |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|------------|----------------------------|------------------|
| 1.                                                                                                                                                                                                                                                                                                                                                                                                      | Aldrin                | 0.05 mg/kg | Leafy Vegetables           | 1997             |
| 2.                                                                                                                                                                                                                                                                                                                                                                                                      | Bifenthrin            | 0.03 mg/kg | Spices, Fruits and Berries | 2011             |
| 3.                                                                                                                                                                                                                                                                                                                                                                                                      | Chlorpyrifos          | NR         | -                          | -                |
| 4.                                                                                                                                                                                                                                                                                                                                                                                                      | Dichlorvos            | 0.1 mg/kg  | Spices                     | 2005             |
| 5.                                                                                                                                                                                                                                                                                                                                                                                                      | Dimethoate            | NR         | -                          | -                |
| 6.                                                                                                                                                                                                                                                                                                                                                                                                      | Fipronil              | NR         | -                          | -                |
| 7.                                                                                                                                                                                                                                                                                                                                                                                                      | Malathion             | 0.5 mg/kg  | Spices, Fruits and Berries | 2005             |
| 8.                                                                                                                                                                                                                                                                                                                                                                                                      | Monocrotophos         | NR         |                            |                  |
| 9.                                                                                                                                                                                                                                                                                                                                                                                                      | Parathion             | 0.2 mg/kg  | Spices, Fruits and Berries | 2005             |
| 10.                                                                                                                                                                                                                                                                                                                                                                                                     | Phorate               | 0.1 mg/kg  | Spices, Fruits and Berries | 2005             |
| 11.                                                                                                                                                                                                                                                                                                                                                                                                     | Phosalone             | 2 mg/kg    | Spices, Fruits and Berries | 2005             |
| 12.                                                                                                                                                                                                                                                                                                                                                                                                     | Triazophos            | 0.07 mg/kg | Spices, Fruits and Berries | 2016             |
| 13.                                                                                                                                                                                                                                                                                                                                                                                                     | $\alpha$ - Endosulfan | 0.5 mg/kg  | Spices, Fruits and Berries | 2005             |
| <p>* There are currently no specific MRLs for Fenugreek Seeds (HS 0782). The MRLs mentioned above are from the corresponding subgroups (Spices, Fruits and Berries) and corresponding groups (Herbs, Leafy Vegetables).</p> <p>* FSSAI MRL for spices and herbs are not Fixed. In Case of Non-Registered (NR) pesticide, default MRL 0.01mg/kg or BDL will apply as per orders dated 08 April 2024.</p> |                       |            |                            |                  |

## Sampling

For the present project random sampling was done from the various markets in Udaipur -Chittorgarh belt local fields and markets. Depending upon the nature of the fenugreek samples were enclosed in a clean blotting paper and wrapped inside a clean, paper envelope. The addition of a small sachet of silica gel to the envelope helps to reduce the moisture content of the system.

## Sample preparation

The acetate-buffered QuEChERS sample preparation method for pesticides (AOAC Official Method 2007.01) was applied to all the samples. After homogenization with a house-hold mill (equipped with stainless steel knives), a 15 g portion of the homogenized sample was weighed into a 50 ml polytetra fluoro ethylene (PTFE) tube and 100 ml of 50 mg/ml triphenyl phosphate (TPP) surrogate standard solution in acetonitrile was added followed by 15 ml of acetonitrile containing 1% acetic acid (v/v not accounting for purity). Then, 6 g MgSO<sub>4</sub> and 2.5 g sodium acetate trihydrate (equivalent to 1.5 g of anhydrous form) were added, and the sample was shaken forcefully for 4 min. The sample was then centrifuged at 4000 rpm for 5 min and 5 ml of the supernatant were transferred to a 15 ml PTFE tube to which 750 mg MgSO<sub>4</sub> and 250 mg PSA were added. The extract was shaken using a vortex mixer for 20 s and centrifuged at 4000 rpm again for 5 min. Approximately 3ml of the supernatant were filtered through a 0.45 mm PTFE filter (13 mm diameter), and 800 ml portions were transferred to autosampler vials. The extracts were evaporated to dryness under a stream of argon and reconstituted in 800 ml acetonitrile/water (20/80, v/v) for the GC-MS analysis.

## Determination of Pesticide Residues by Gas Chromatography Mass Spectrometry

Determination of pesticides was carried out by gas chromatography with an autosampler and tandem mass spectrometry. The GC separation was performed on a TSQ Endura capillary column of 30 m length, 0.25 mm internal diameter and 0.25 µm film thickness, supplied by Phenomenex. The oven conditions were set as follows: 70 °C, then 70–230 °C at 10 °C min<sup>-1</sup>, hold for 1 min, 230–270 °C at 5 °C min<sup>-1</sup> and finally 270–310 °C at 10 °C min<sup>-1</sup>, hold for 3 min. The total run time was 32 min. The inlet temperature was set to 250 °C. Helium was used as a carrier the chromatographic analysis with flow of 1.0 mL min<sup>-1</sup> and constant carrier gas pressure control. The injection volume of 3 µL was selected for all analyzes in splitless mode at higher pressure conditions (150 kPa). The triple quadrupole mass spectrometer in electron impact (EI) ionization mode was operated with a 70 V ionization voltage and 150 µA of emission current. Argon gas at a pressure of 200 kPa was chosen as the collision gas. The interface (transfer line to the tandem MS), ion source and quadrupole temperatures were maintained at 310 °C, 230 °C and 150 °C, respectively. The overall run duration was 10 min. Comparing the retention duration of the matrix-matched calibration standard allowed for identification and the calibration curve created with the matrix-matched calibration standard was used for quantification.

## Statistical analysis

The acquired data was subjected to ANOVA and test of significance at the significance levels of  $P < 0.001$ , 0.01 and 0.05.

## Observations

| Table: 2 Quantification of Different Pesticidal Residue Via GC-MS Found in Fresh Green Seeds of <i>Trigonella foenum-graecum</i> Obtained from Various Markets of Udaipur-Chittorgarh Belt |            |                |       |     |                  |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|----------------|-------|-----|------------------|
| S.No                                                                                                                                                                                       | Area       | Pesticide      | RT    | PA  | PR Conc. (mg/kg) |
| 1.                                                                                                                                                                                         | Mangalwar  | Bifenthrin     | 11.04 | 222 | 0.032 ± 0.04     |
|                                                                                                                                                                                            |            | Chlorpyrifos   | 15.26 | 212 | 0.029 ± 0.02     |
|                                                                                                                                                                                            |            | Aldrin         | 13.47 | 327 | 0.021 ± 0.05     |
|                                                                                                                                                                                            |            | Triazophos     | 17.67 | 129 | 0.13 ± 0.006     |
|                                                                                                                                                                                            |            | Monocrotophos  | 9.46  | 113 | 0.07 ± 0.021     |
| 2.                                                                                                                                                                                         | Kapsan     | Parathion      | 17.86 | 187 | 0.41 ± 0.036     |
|                                                                                                                                                                                            |            | Phorate        | 7.67  | 147 | 0.009 ± 0.001    |
|                                                                                                                                                                                            |            | Malathion      | 12.17 | 178 | 0.13 ± 0.12      |
|                                                                                                                                                                                            |            | Fipronil       | 7.65  | 277 | 0.11 ± 0.004     |
|                                                                                                                                                                                            |            | Phosalone      | 18.79 | 318 | 0.28 ± 0.021     |
| 3.                                                                                                                                                                                         | Fatehnagar | Dimethoate     | 17.56 | 384 | 0.05 ± 0.013     |
|                                                                                                                                                                                            |            | Dichlorvos     | 17.22 | 339 | 0.006 ± 0.002    |
|                                                                                                                                                                                            |            | Parathion      | 18.10 | 269 | 0.22 ± 0.001     |
|                                                                                                                                                                                            |            | Triazophos     | 18.43 | 222 | 0.08 ± 0.002     |
|                                                                                                                                                                                            |            | Phorate        | 8.78  | 106 | 0.16 ± 0.13      |
|                                                                                                                                                                                            |            | Chlorpyrifos   | 14.71 | 498 | 0.009 ± 0.004    |
|                                                                                                                                                                                            |            | Fipronil       | 6.52  | 234 | 0.24 ± 0.04      |
| 4.                                                                                                                                                                                         | Dabok      | Aldrin         | 14.80 | 363 | 0.04 ± 0.011     |
|                                                                                                                                                                                            |            | Monocrotophos  | 9.57  | 280 | 0.16 ± 0.018     |
|                                                                                                                                                                                            |            | Bifenthrin     | 13.66 | 176 | 0.019 ± 0.01     |
|                                                                                                                                                                                            |            | Phosalone      | 19.31 | 188 | 0.44 ± 0.21      |
|                                                                                                                                                                                            |            | Dimethoate     | 17.97 | 327 | 0.04 ± 0.007     |
|                                                                                                                                                                                            |            | α - Endosulfan | 14.49 | 198 | 0.007 ± 0.002    |
| 5.                                                                                                                                                                                         | Mavli      | Dichlorvos     | 16.47 | 248 | 0.002 ± 0.001    |
|                                                                                                                                                                                            |            | Malathion      | 12.20 | 298 | 0.018 ± 0.015    |
|                                                                                                                                                                                            |            | Chlorpyrifos   | 16.02 | 498 | 0.03 ± 0.004     |
|                                                                                                                                                                                            |            | Aldrin         | 14.37 | 420 | 0.07 ± 0.011     |
|                                                                                                                                                                                            |            | Bifenthrin     | 11.87 | 368 | 0.023 ± 0.035    |
|                                                                                                                                                                                            |            | Parathion      | 17.23 | 355 | 0.10 ± 0.008     |
| RT: Retention Time is expressed in minutes and PA: Peak Area in Volt-minutes.                                                                                                              |            |                |       |     |                  |

## Result and Discussion

## Result

The quantity of pesticides identified in each sample from five separate markets in the Udaipur-Chittorgarh region varied between 1 and 13 pesticides. Samples from Kapsan and Mangalwar indicated the detection of five pesticides, whereas samples from Dabok and Mavli revealed the presence of six pesticides. Dimethoate, fipronil, monocrotophos and chlorpyrifos are among the 13 pesticides that are present in fresh green fenugreek seeds but are not registered with the CODEX Alimentarius Commission. In comparison to their respective MRLs, CAC finds that the concentrations of aldrin, chlorpyrifos, parathion, triazophos,

monocrotophos, dimethoate, phorate, and fipronil are greater. Except for triazophos, monocrotophos, dimethoate, and fipronil, their concentrations are not higher in all the areas they are found. The results indicated that samples collected from the Fatehnagar market contained a higher quantity of pesticides compared to samples from other markets. The highest residue detected in Dabok samples for phosalone was  $0.44 \pm 0.21$  mg/kg (Table-2), whereas the lowest residue recorded was  $0.002 \pm 0.001$  mg/kg of Dichlorvos from Mavli region samples. From the results presented in table-2, one can observe a range of concentrations of various pesticides throughout the Udaipur-Chittorgarh region. The residues of Aldrin (prohibited) were also detected in the samples from Mangalwar, Dabok, and Mavli. This may be because of its longevity in the environment for ten years or longer, as organochlorines are persistent organic pollutants. Parathion, prohibited in numerous countries because of its highly toxic effects, was also detected in the samples collected from Kapasan, Fatehnagar, and Mavli.

## Discussion

Research conducted on rivers and surface waters in the Cienega region of Jalisco, Mexico, revealed that Malathion concentrations ranged from  $311.76 \mu\text{g L}^{-1}$  to  $717.34 \mu\text{g L}^{-1}$  in rivers and  $771.49$ – $863.49 \mu\text{g L}^{-1}$  in surface waters (Silva-Madera et al., 2021). Their findings showed that levels of Malathion exceeded the WHO guideline threshold. Several studies revealed that pesticides resulted in reproductive organ harm in other species such as mice. Mouse ovaries exhibited lower ovarian weight and fewer types of follicles after exposure to MCP. This research revealed follicular wall damage and oocyte atresia in the ovaries of all MCP treated groups, along with follicular wall damage, atresia and vacuolation in the ovaries of all GLY treated groups after 30 days of exposure (He et al, 2023). Findings from ElSheikh et al. (2022) indicated that 36% of samples, both fruits and vegetables, were free of pesticides, whereas 64% contained between one and 20 pesticide residues. Cucumber, pepper, zucchini, and tomato displayed 15, 12, 12, and 10 pesticide residues, respectively. In the fruit samples, apple, grapes, and apricot had 20, 18, and 14 pesticide residues, respectively. Findings of Momtaz and Khan in (2024) showed Chlorpyrifos residue in 86.67% of samples from the field (site-2), and 84.62% of these samples showed chlorpyrifos levels exceeding the MRL. Chlorpyrifos residue was detected in 64.44% of samples obtained from local markets, with 55.17% of the positive samples showing chlorpyrifos levels exceeding MRL. A study of Aliomrani et al. (2021) demonstrated that the immobility times were increased with Pln doses of 6, 12, and 40 mg/kg ( $185 \pm 17$  s,  $186 \pm 9$  s,  $172.0 \pm 7$  s respectively) in comparison to control animals ( $149 \pm 8$  s,  $p < 0.01$ ). Additionally, by day 6, following multiple Pln exposures (0.6, 6, 12, 20 mg/kg), the immobility times were greater than the control ( $190 \pm 20$ s,  $210 \pm 4$  s,  $196 \pm 10$ s,  $204 \pm 9$  s respectively, versus control  $153 \pm 7$ ,  $p < 0.001$ ). A study of An et al. (2024) indicate that sublethal concentrations of bifenthrin (LC1, LC10, LC20 and LC30) substantially decrease sex pheromone production and mating success in a dose-dependent fashion. Additionally, these sublethal exposures affect the expression of neuropeptides that activate pheromone biosynthesis and important juvenile hormone signaling genes, such as methoprene-tolerant and Krüppel-homologue 1. Activity assays of enzymes and measurements of metabolites showed that sublethal exposure to bifenthrin lowers trehalose and pyruvic acid levels, inhibiting the enzyme activities necessary for the

synthesis of sex pheromones. Moreover, exposure to bifenthrin postpones ovarian maturation, diminishes ovary size and lowers egg production and hatch rates.

Pesticide monitoring initiatives aim to ensure that MRLs in agricultural goods stay within government-approved limits, and that pesticides are not improperly used, potentially resulting in unanticipated residues in food. Various analytical techniques have been created and validated globally for detecting pesticide residues in spices and a significant challenge in creating extraction methods for these residues is the intricate matrix composition and elevated levels of co-extractives that must be considered for accurate results (Mathew et al. 2023).

## Conclusion

For years, agrochemicals such as fertilizers and pesticides have been utilized to enhance agricultural productivity and safeguard crops. The overuse of these chemicals has certain harmful effects on the environment. Their extended residual effect has driven us to search for eco-friendly biological resources that can maintain crop pests and diseases within a threshold limit. Among various biological resources, microorganisms are the essential bioagents currently employed in protecting crops. Microorganisms enhance plant growth by inhabiting the plant's rhizosphere and providing essential nutrients (nitrogen and phosphorus) necessary for growth. Different pests and pathogens are managed using microbes and their additives. The destiny of the pesticides must be assessed in leafy vegetables to determine their safe application. Additionally, it is necessary to investigate methods to minimize its residues in leafy greens once the pests have been managed. Arka Microbial Consortia (AMC) created at IIHR has demonstrated outstanding outcomes in enhancing the vegetative growth of vegetables, supporting the idea that it can also lower pesticide residues in leafy vegetables through residue 'dilution'. Likewise, household practices such as washing and cooking might minimize the residues of these pesticides, but the degree of this reduction remains unknown. Consequently, experimental data will be produced to assess the decrease of residues in leafy greens by utilizing the methods mentioned above. To minimize the improper use of chemical pesticides in vegetables, implementing alternative pest management strategies is essential. Biopesticides are tailored for specific pests and provide an environmentally friendly and efficient answer to pest issues. Even with various limitations, biopesticides are utilized in vegetable farming systems. They present a reduced risk to both the environment and human health.

## Conflict of Interest

No potential conflict of interest

## Fundings

None

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