Systematic Evaluation of Pesticide Residue Dynamics and their Influence on Biochemical Constituents and Antidiabetic Efficacy of Fenugreek

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Abstract

Fenugreek (*Trigonella foenum-graecum* L.) is an established medicinal plant having substantial therapeutic applications, particularly in the prevention and treatment of type II diabetes mellitus. Fenugreek seeds, which are traditionally used in Indian cuisine, contain bioactive compounds such as diosgenin, galactomannan and 4-hydroxyisoleucine, which contribute to their hypoglycemic properties and metabolic benefits by modulating alpha-amylase and alpha-glucosidase, the key enzymes involved in metabolising carbohydrates and glucose absorption. However, the legacy of the Green Revolution introduced extensive use of synthetic pesticides, notably organochlorines and organophosphates, which led to residue accumulation in agricultural environments and crop matrices.

In this context, our study conducts a targeted investigation of fenugreek samples retrieved from local marketplaces in Udaipur, Rajasthan, in order to investigate the residue of pesticides and its impact on medicinal efficacy. We measured pesticide residues and linked their presence to alterations in the plant's bioactive content using modern analytical methods. Anti-diabetic experiments were carried out to explore the inhibitory effects on alpha-amylase and alpha-glucosidase, finding significant diversity related to residue patterns. These findings highlight the possible influence of agricultural contaminants on fenugreek enzymatic modulation and antidiabetic activity.

As agriculture transitions toward sustainable models, reconciling productivity with ecological stewardship is essential. Investigating the correlation between pesticide residues and alterations in fenugreek's bioactive constituents and enzyme-regulating potential provides critical insights into preserving its antidiabetic potency. This research advocates for the integration of residue screening protocols in medicinal plant evaluation and the implementation of reduced-pesticide farming systems. Such initiatives are vital for safeguarding food safety, pharmacological efficacy, and ecological balance, supporting a future where therapeutic reliability and sustainable food systems coexist.

Keywords: *Trigonella foenum-graecum,* diabetes mellitus II, alpha- amylase, alpha- glucosidase, organophosphate, organochlorine

1. Introduction

Food safety is a major problem globally as a consequence of the increased need for food production spurred on by global population growth. Authorities emphasise expanding food production as worldwide population soars to nearly 10 billion people by 2050. An estimated 97 million individuals are added to the world's population annually. In order to sustain the growing world population, food output will have to increase by 70% by 2050 (Anani et al. 2020). In an effort to fulfil food demands while using the same limited resources, such as land, water, and other natural assets, the current agricultural system is under a lot of strain due to the expanding population (Khan et al. 2023). With the objective to boost the productivity of crops, the utilisation of herbicides, fungicides, insecticides, pesticides, fertilisers, nematicides, and soil amendments is gaining prominence.

The 'Green Revolution' has been discovered to be reliant on pesticides. The developments in modern agriculture have led to a greater prevalence of the pesticide application in the production and growing of agricultural products. For many developing nations, particularly those in tropical regions aiming to expand their economies and thrive in the global market, pesticides have become an essential tool. These countries are able to provide temperate-climate countries with product that would otherwise be inaccessible by allowing them to cultivate fresh fruits and vegetables all year long, even during the off-season. As a result, they are increasingly playing a crucial role in the production of food worldwide, bolstering local economies and international commerce while producing many crop harvests year. Due to the improper use and illegal production of high-toxicity pesticides, the extensive usage of the pesticide industry in agriculture has resulted in excessive chemical residues (Leskovac and Petrovic, 2023).

Vegetables have a crucial role in maintaining health and preventing disease in Indian subcontinent. Indian meals contain around 150-250 grammes of veggies every day. India has a bounty of green leafy vegetables that are suitable for temperate, tropical, and subtropical regions that are cultivated throughout the year (Noopur et al. 2023). While numerous green vegetables are cultivated in India, only a handful few—amaranth (*Amaranthus spp*.), fenugreek (*Trigonella foenum-graecum*), drumstick leaves (*Moringa oleifera*) and Indian spinach (*Basella spp*.) have acquired commercial significance and are extensively farmed (Farha et al. 2018). In India, Rajasthan is a prominent grower of fenugreek, making up a sizable amount of the country's total acreage and output. Sikar, Chittorgarh, Jaipur, Pali, and Jhalawar are some of the main districts in Rajasthan that cultivate fenugreek (Meena et al. 2024). Known as "poverty man's vegetables" because of its high yield potential and inexpensive production costs, GLVs are among the most reasonably priced vegetables on the market. Incorporating GLVs into the diet will help to eliminate malnutrition within society since they are an affordable source of nutrients (Yadav et al. 2018).

Fenugreek has a wide range of applications, from religious to gastronomic, and these applications are frequently rooted in liturgy. Fresh fenugreek leaves and stems are often prepared as a winter vegetable in India, but the seeds are utilised all year long to add aroma to a variety of cuisines (Aasim et al. 2018). Additionally, fenugreek seeds are used medicinally and consumed uncooked as sprouts. Green, fresh leaves are utilised in curries and rolled into flatbread. The plant's fresh leaves, which are frequently marketed as methi, can be tossed into cooked meals and salads. Leaves preserve much of their flavour when dried, and they're great as last-minute upgrades to soups, stews and curries (Sarwar et al. 2020). Fenugreek is known for its potential benefits in managing diabetes. It contains soluble fibre, which helps slow down digestion and absorption of carbohydrates, leading to lower blood sugar levels. Studies suggest that fenugreek may improve glucose tolerance and reduce bad cholesterol, making it beneficial for both type 1 and type 2 diabetes (Kim et al. 2023).

However, pesticide residues in vegetables are influenced by both internal transport mechanisms and external environmental factors. These residues can accumulate in the fatty tissues of consumers through dietary exposure via the food chain, and they also build up in crops and soil, forming complex intermediates and metabolites (Li 2020). These are referred to as "emerging contaminants" and have garnered more attention because to the significant harm they cause to both the environment and human health. According to some research, using pesticides may increase the risk of teratogenicity, diabetes, cancer, foetal mortality, headaches, allergies, depression and respiratory disorders (Pattnaik et al. 2020).

Determining the level of pesticide residues in fenugreek samples in and around southern regions of Rajasthan, India, is crucial in light of the aforementioned and to evaluate the current environmental load of chemical residues. The Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) methodologies are additionally employed in this research to estimate the pesticides in fenugreek that is purchased from the local market in the Udaipur, Chittorgarh belt region. This study also throws light on the subsequent therapeutic efficacy of pesticide contaminated fenugreek in controlling diabetes.

2. Material and Methods

2.1 Sample Procurement

In this investigation, fresh fenugreek samples were collected at random from local markets and farms in the Udaipur-Chittorgarh area and surrounding districts. Samples weighing 100 g were collected from each market location and sealed in polyethylene bags. The sample packets were labelled before being delivered to the laboratory in an ice box. The samples were refrigerated until extraction.

2.2 Sample Extraction

The QuEChERS technique was adopted to extract pesticides from all fenugreek samples. The samples were homogenised using a homogeniser within 24 hours of collection. 1 gm sodium sulphate, 20 ml acetonitrile, and 1 gram salt mixture were added to the homogenised sample. The sample was transferred into 50 mL centrifuge tubes and subjected to vigorous shaking for 2 minutes to ensure thorough mixing. Following this, the sample underwent centrifugation at 4000 rpm for 4 minutes, after which the supernatant was carefully collected and transferred to a polytetrafluoroethylene (PTFE) purification tube. To facilitate purification, 750 grams of magnesium sulphate ($MgSO_4$) and 250 grams of primary secondary amine (PSA) were added to the solution. The mixture was then vortexed for 1 minute to enhance homogeneity and subsequently centrifuged again at 4000 rpm for 5 minutes. The resulting supernatant was filtered through a PTFE mesh to remove residual particulates and was finally transferred into 10 mL autosampler vials for subsequent analysis via gas chromatography coupled with mass spectrometry (GC-MS).

2.3 Identification and Quantification of Pesticide Residues by Gas Chromatography Mass Spectrometry

A gas chromatograph was used in conjunction with a mass spectrometer to identify the pesticide compounds. The analysis was done on TSQ Endura GC-MS equipped with MSD and capillary column of 30 m length, 0.25 µm film thickness, and 0.25 µm internal diameter, paired with autosampler. The column temperature was first fixed at 90°C for 2 minutes. The temperature was raised to 260°C for 5 minutes. The injection port temperature was kept at 250°C. Helium was used as the carrier gas for separation, with a flow rate of 2 ml/min and a velocity of 35 cm/s. The injection volume was set at 1µl, and the Gc was operated in splitless mode at higher column pressure (150 kPa). The interface temperature of the equipment was maintained at 350°C. The overall run duration was 40 minutes. The triple quadrupole mass spectrometer used electron impact (EI) ionisation mode with a voltage of 70 V and an emission current of 150 µA. Argon gas at a pressure of 200 kPa was selected as the collision gas. Temperatures were kept at 310 ◦C for the interface, 230°C for the ion source, and 150°C for the quadrupole. The whole run time was 10 minutes. Standards were evaluated over the course of the analysis. A total of 20 samples were examined for each sample type and the analysis was conducted in duplicate. Identification was made attainable by comparing the matrix-matched calibration standard's retention time, and quantification was done using the calibration curve that was produced using the matrix-matched calibration standard.

2.4 In- vitro analysis of Anti α-Amylase activity

The alpha amylase inhibitory assessment was performed using an amylase activity test kit (MAK009) sourced from Sigma Aldrich solutions. To conduct the α- amylase inhibition test, a reaction mixture was generated by combining 200µl of 0.02 sodium phosphate buffer, 20µl of enzyme, and plant extract in various solvents. The mixture was allowed to incubate at room temperature for 10 minutes. The solution was prepared by adding 2µl of starch and 400 µl of DNS reagent, then immersed in a water bath for 5 minutes. After cooling, the solution's absorbance at 540 nm was measured using a spectrophotometer. The control solutions were made without the inclusion of plant extract and served as blanks.

$$\mathbf{Inhibition}\ (\%) = \frac{Abs540\ (control) - Abs540\ (extract) * 100}{Abs540(control}$$

2.5 In -vitro analysis of Anti α-glucosidase activity

The assessment of anti α- glucosidase activity was performed using glucosidase activity assay kit (MAK123) procured from Sigma Aldrich solutions. 1 mg of α-glucosidase enzyme was dissolved in 100ml phosphate buffer containing 200mg of bovine serum albumin and the pH of solution was adjusted to 6.8. This solution was used as enzyme extract. The working solution of plant extract was premixed with 490µl of phosphate buffer (Ph6.8) and 250µl of p-nitrophenyl α-D-glucopyranoside. This mixture was then incubated at 35° C for 5 mins. The enzymatic solution of α- glucosidase was added to this solution and incubated for 37° C for 15 mins. The reaction was terminated by the addition of 2000 µL Na_2CO_3 200 mM. α-glucosidase activity was determined spectrophotometrically at 410 nm on spectrophotometer. Acarbose was used as positive control of α-glucosidase inhibitor.

$$\textbf{Inhibition}\ (\%) = \frac{Abs410\ (control) - Abs410\ (extract) * 100}{Abs410(control}$$

2.6 Statistical Analysis

The data was analysed using ANOVA and tested for significance at P-values ≤ 0.001, 0.01 and 0.05.

3. Observations

Table 1: GC-MS Determination of Pesticide residue in tender leaves of Fenugreek obtained from local market places of Udaipur- Chittorgarh belt

S. No	Area	Pesticide Name	RT (in mins)	PA	Calculated Amount (mg/kg)
1.	Bansen	Chlorpyrifos	18.50	286	0.023±0.04
		Dimethoate	14.82	357	0.17±0.011
		α-Endosulfan	18.32	267	0.006±0.008
		Malathion	7.34	198	0.24±0.006
		Permethrin	29.46	280	0.008±0.04
2.	Chittorgarh	Dichlorvos	9.35	353	0.005±0.002
		Dimethachlor	8.32	298	0.003±0.001
		Imidacloprid	9.38	253	0.042±0.02
		Malathion	8.40	184	0.16±0.012
		Parathion	18.20	278	0.15±0.08
3.	Mavli	Aldrin	8.55	285	0.035±0.02
		Cypermethrin	25.34	469	0.071±0.05
		Dichlorvos	10.70	468	0.003±0.001
		Fenthion	20.46	136	0.010±0.001
		Imidacloprid	8.59	266	0.047±0.032
4.	Nimbahera	Chlorpyrifos	16.98	152	0.028±0.013
		Dimethoate	16.84	338	0.23±0.03
		Heptachlor	14.35	442	0.051±0.02
		Imidacloprid	10.55	287	0.038±0.01
		Permethrin	30.58	139	0.04±0.02

5.	**Jhadol**	Cypermethrin	23.61	307	0.04±0.02
		Dichlorvos	9.23	421	0.004±0.02
		Heptachlor	13.90	365	0.16±0.02
		Permethrin	30.42	126	0.08±0.03
		Phorate	9.47	249	0.011±0.002
		Phosalone	17.59	179	0.04±0.01
6.	**Phalasiya**	Bifenthrin	10.42	239	0.006±0.002
		Chlorpyrifos	15.61	115	0.06±0.03
		Fenthion	20.38	250	0.05±0.003
		Imidacloprid	11.27	222	0.029±0.001
		Phorate	13.49	274	0.014±0.11
7.	**Sarada**	Aldrin	9.25	420	0.072±0.05
		Cypermethrin	21.40	310	0.05±0.005
		Dimethoate	16.57	114	0.25±0.01
		Heptachlor	15.46	359	0.03±0.01
		Malathion	12.27	254	0.49±0.15
		Phosalone	18.40	186	0.052±0.02
8.	**Vallabhnagar**	Chlorpyrifos	16.52	124	0.049±0.02
		Dimethoate	17.48	197	0.4±0.05
		Fenthion	23.74	356	0.025±0.002
		Malathion	11.13	165	0.43±0.03
		Parathion	19.75	355	0.22±0.16

Table 2: Assessment of detected pesticide residue in comparison to Maximum residue limits (MRL)

S. No.	Pesticide Name	Pesticide Residue Range (Min- Max.)	MRL (mg/kg)	Exceeds MRL
1.	Aldrin	0.035 - 0.072	0.01	Yes
2.	Bifenthrin	0.006	0.02	No
3.	Chlorpyrifos	0.023 – 0.06	0.05	Yes
4.	Cypermethrin	0.04 – 0.071	0.01	Yes
5.	Dichlorvos	0.003 – 0.005	0.15	No
6.	Dimethoate	0.17 – 0.4	0.1	Yes
7.	Dimethachlor	0.003	0.1	No
8.	Fenthion	0.01 – 0.05	0.01	Yes
9.	Heptachlor	0.03 – 0.16	0.01	Yes
10.	Imidacloprid	0.029 – 0.047	0.40	No

11.	Malthion	0.16 – 0.49	0.15	Yes
12.	Parathion	0.15 – 0.22	0.01	Yes
13.	Permethrin	0.008 – 0.08	0.01	Yes
14.	Phorate	0.011 – 0.014	0.025	No
15.	Phosalone	0.04 - 0.052	0.1	No
MRL: Maximum residue limit (in mg/kg)				

Table 3: Evaluation of anti-diabetic enzyme activity of fenugreek samples obtained from various local marketplaces of Udaipur- Chittorgarh region

S.No	Region of Fenugreek Sample	α-amylase (% inhibition)	α-glucosidase (% inhibition)
1.	Bansen	42.62±0.15	41.28±0.18
2.	Chittorgarh	39.37±0.21	40.42±0.25
3.	Mavli	40.56±0.24	39.27±0.12
4.	Nimbahera	39.63±0.18	38.15±0.25
5.	Jhadol	38.64±0.33	40.35±0.24
6.	Phalasiya	44.14±0.21	46.15±0.40
7.	Sarada	37.12±0.28	34.50±0.39
8.	Vallabhnagar	35.58±0.25	36.62±0.25
The enzyme inhibition of α- amylase and α-glucosidase is expressed as % inhibition.			

4. Result

The GC-MS profiling of tender fenugreek leaves from eight marketplaces in the Udaipur-Chittorgarh area revealed diverse pesticide residue patterns, with significant heterogeneity in component type and concentration. The most frequently discovered organophosphates were malathion and dimethoate, which were found in five and six places, respectively, with maximum values of at Sarada) and Vallabhnagar. Imidacloprid was found consistently at five locations, indicating its systemic application. Concerns over environmental persistence were raised by the presence of organochlorines as Heptachlor and Aldrin in Jhadol and Sarada, respectively. Among all compounds, Malathion, Dimethoate, and Imidacloprid were the most prevalent, while Dimethachlor and Dichlorvos showed limited occurrence (≤0.005 mg/kg), detected only in single locations. Sarada and Jhadol each had six different pesticides identified showing the largest residue variety. In comparison to codified MRLs, these results highlight the geographical variation in pesticide use and bolster the necessity of region-specific residue monitoring.

Compliance with safety standards (MRL) was evaluated for the pesticide residue in fenugreek samples. The safety of ingestion is called into question since levels of many pesticides, including Aldrin, Chlorpyrifos, Cypermethrin, Dimethoate, Fenthion, Heptachlor, Malathion, Parathion, and Permethrin, were found to be higher than their MRL. Some pesticides, such as Bifenthrin, Dichlorvos, Dimethachlor, Imidacloprid, Phosalone and Phorate, were identified at concentrations below their maximum recommended level (MRL), which may not constitute a serious health concern. The highly lethal chemicals parathion, heptachlor and aldrin surpass safety limits. Because certain pesticides exceed their maximum recommended levels, the tainted fenugreek could not be deemed safe for human consumption.

The comparative assessment of pesticide residues and antidiabetic enzyme inhibition across fenugreek samples suggests an inverse relationship between residue burden and bioactivity potential. The pesticide concentrations of imidacloprid and chlorpyrifos were comparatively lower in Phalasiya, which exhibited the strongest inhibition of α-amylase and α-glucosidase. In contrast, Vallabhnagar, which had the lowest α-amylase inhibition, had much more organophosphate residues, especially dimethoate and malathion, which probably reduced the enzymatic efficacy by compromising the integrity of the phytochemicals. Likewise, higher levels of organophosphates, especially malathion, were linked to Sarada and Vallabhnagar, which showed the lowest α-glucosidase inhibition. These results suggest that increased pesticide deposition could disrupt the stability or expression of bioactive substances that inhibit enzymes. Other medicinal plants subjected to agrochemicals have shown similar patterns, indicating the necessity of integrated residue–bioefficacy studies.

5. Discussion

The present study underscores the efficacy of Gas Chromatography-Mass Spectrometry (GC-MS) in detecting pesticide residues in leafy vegetables, reaffirming its status as a gold standard in analytical chemistry for food safety assessments. GC-MS offers high sensitivity and specificity, making it particularly suitable for identifying and quantifying volatile and semi-volatile pesticide compounds in complex plant matrices. Recent investigations have highlighted the prevalence of pesticide residues in various vegetables. For instance, a study conducted in Bangladesh analyzed chlorpyrifos residues in cauliflower, cabbage, and eggplant using GC-MS. The findings revealed that 44% of cauliflower samples, 68% of cabbage samples, and 80% of eggplant samples contained chlorpyrifos residues, with a significant proportion exceeding the Maximum Residue Limits (MRLs) set by regulatory authorities (Momtaz and Khan 2024). Similarly, research from India reported the presence of organophosphorus pesticides like chlorpyrifos and carbamates such as carbaryl in vegetables like brinjal, ladyfinger, tomato, chili, and cabbage, with concentrations surpassing permissible limits (Khan et al. 2021).

These findings are alarming, considering the potential health risks associated with chronic exposure to pesticide residues, including neurological disorders, hormonal imbalances, and increased cancer risk. The detection of such residues in commonly consumed vegetables emphasizes the need for stringent monitoring and regulation of pesticide use in agriculture.

Methodologically, the application of the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) extraction method combined with GC-MS has proven effective for multi-residue analysis in leafy vegetables. A study from Turkey employed this approach to analyze 363 pesticides in dill, rocket, and parsley, achieving satisfactory recoveries and precisions for all residues (Deveci et al. 2023). The integration of QuEChERS with GC-MS not only streamlines the sample preparation process but also enhances the accuracy and reliability of the analytical results.

Beyond the immediate concerns of food safety, emerging research has explored the potential link between pesticide exposure and the development of metabolic disorders, particularly type 2 diabetes mellitus (T2DM). A systematic review and meta-analysis encompassing 19 observational studies with a total of 45,813 participants found a significant association between pesticide exposure and increased risk of T2DM. Specifically, exposure to organochlorine and pyrethroid pesticides was associated with higher odds of developing T2DM (Chen et al. 2025).

Additional studies have corroborated these findings. A study in a rural Korean population found that pesticide exposure was linked to an increased prevalence of diabetes, particularly among overweight or obese individuals (Park et al. 2019). These epidemiological findings are supported by toxicological studies demonstrating that certain pesticides can disrupt endocrine function and glucose metabolism, potentially leading to insulin resistance and impaired glucose tolerance. For instance, elevated levels of β-hexachlorocyclohexane (β-HCH), dieldrin, and p,p'-dichlorodiphenyldichloroethylene (p,p'-DDE) have been associated with increased fasting and postprandial glucose levels, as well as higher insulin resistance (Evangelou et al. 2016).

6. Conclusion

The pesticide residue burden and antidiabetic enzyme activity of fenugreek samples from the Udaipur-Chittorgarh area fluctuated across location. In Sarada and Vallabhnagar, higher concentrations of organophosphates such dimethoate and malathion led to lower inhibition of α-amylase and α-glucosidase. In contrast, Phalasiya demonstrated greater enzyme inhibition at lower pesticide doses. The observed inverse correlation between pesticide residues and antidiabetic enzyme inhibition in fenugreek underscores the urgent need for enhanced residue monitoring in medicinal plant supply networks. These results emphasise the necessity of integrated safety evaluations in phytopharmacological applications by indicating that pesticide exposure may reduce the bioactive effectiveness of medicinal plants.

Conflict of Interest

The authors declare that they have no conflict of interest that could have influenced the work reported in this research paper.

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