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Pakistan Academy of Sciences

Letter to the Editor

Balancing Delay and Demise: Peptides and Leaf Senescence

Rishan Singh*

Research Media SR & Golden Key International, KwaZulu-Natal, Durban, 4001, South Africa

Leaf senescence refers to the final stage of leaf development whereby a leaf undergoes biochemical, physiological and structural changes. During the process of leaf development, a leaf gradually undergoes a regulated form of aging, which is characterized by the yellowing of leaves, the translocation of valuable resources to developing seeds and/or young leaves, as well as, deterioration of metabolic activity. Leaf senescence is programmed and controlled not only by genes, but also influenced by environmental factors and hormones, such as ethylene, abscisic acid, and cytokinins [1]. Recently, Li *et al.* [2] reported that small peptide signals regulate the beginning and end of leaf senescence. In their study, Li *et al.* [2] demonstrated that secreted peptides, and not just hormones and/or transcriptional factors, are indeed mobile messengers that control leaf lifespan. CLEs, IDLs, Peps, PDKs, and SCOOPs, were used as examples to show how peptide signalling creates a homeostatic balance between inhibiting or facilitating senescence.

Zhang *et al.* [3] stated that the CLE14 peptide delays age-dependent and stress-induced senescence by aiding in JUB1-mediated reactive oxygen species (ROS) scavenging, however, Zhang *et al*. [4] reported that the CLE42 peptide induces an antagonistic effect on ethylene signally processes, causing the postponement of aging. In addition, it has been found that leaves exposed to natural light, such as sunlight and darkness, undergo accelerated senescence induced by the IDLE peptide [5]. The above examples highlight how different peptide signals balance the delay and facilitation of senescence, and Li *et al.* [2] thus highlights that peptides are targets for crop improvement strategies. Another recent example where peptides aid in senescence is found in rose plants [6]. In rose plants, petal abscission, a process related to senescence due to organ detachment and nutrient mobilization, occurs through RbIDL peptide signalling [6].

According to Butenko *et al.* [7] and Czyzewicz *et al*. [8], the use of IDL and CLE peptides display a mixture of divergent and convergent roles on the different parameters associated with ROS activity, as well as, the hormonal regulation of senescence. This indicates that those peptides intersect the functionality of gene sets, proteins, as well as, the pathways that produce, sense, and detoxify ROS [9]. Additionally, Kim *et al.* [10] reported a network of hormone biosynthesis, transport, perception, and signally genes - such as jasmonic acid and salicylic acid (senescence-promoting) - and auxin and gibberellins (senescence-delaying), which may possibly intersect the functionality of IDL and CLE peptides. However, as leaf senescence regulation cannot be compartmentalized because it is a complex process, Matsubayashi and Sakagami [11] highlight the conserved function of the PDK peptide in delaying stress-induced senescence by promoting cellular survival, while IDLs and CLEs still implement regulatory control. This emphasizes the balancing act that small peptides play in performing regulatory control over leaf senescence mechanisms. This result shows the intersection of communication networks that have a promising effect on the agricultural sector, specifically because strategic manipulation of peptide signaling could probably improve crop productivity, nutrient mobilization, and stress resilience [12]. There are, nevertheless, still

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* Corresponding Author: Rishan Singh <rishansingh18@gmail.com>

substantial gaps in how senescence is regulated in leaves by peptides, particularly, because peptide-receptor pairs are poorly characterized, i.e., the receptor-ligand interactions involved in activating downstream biological processes are not well understood [13]. Although progress has been slow, a more collaborative effort has been made to grasp the mechanistic links between peptide perception and downstream transcriptional reprogramming in senescence [14]. Further investigation is required to understand the crosstalk between peptide-mediated pathways and classical hormones [15]. Olsson *et al.* [16] recommend the need for comparative studies across plant species under environmental stress conditions, e.g., in field experiments, to examine the intersection between conserved and lineage-specific peptide tasks.

Physiological and ecological understanding of leaf senescence points to a developmental programme, or a plants' response to environmental conditions, however, Li *et al.* [2] showed that senescence needs to be reframed as a process governed by intracellular cues. According to Li *et al.* [2], these cues are dialogues that may either prolong leaf longevity, or expedite its death. By being able to respect the duality afforded by intracellular messengers, the core agricultural challenge of understanding crops aging and their translational potential becomes clearer [17, 18]. This enables us to gather a varied view on plant productivity and resilience, particularly because senescence is more generally known from a hormonal perspective, implying that peptide signals may add to the dimensions of views pertaining to leaf senescence.

Some natural stressors that can be used to envisage the delay or demise of leaf senescence with signaling peptides include fluctuating temperatures, variable water availability, and diverse microbial communities. Additionally, comparative studies can offer evolutionary insights, particularly since individual plants must adapt to their growth environment in order to grow and thrive properly [16]. Furthermore, understanding the diversity and specificity of peptide functions tie in with their acclimatization resilience to tolerate stressors, and the possibility to thrive with better growth characteristics [16]. Examples of comparative peptide studies that could be investigated are: (1) cross-species transcriptomics analysis amongst different field crops, e.g., barley, rice, etc. (2) comparative analysis of small secreted peptide signaling in vascular and non-vascular plants, and (3) comparative transcriptomics of multi-stressor responses [19], e.g., using cold-, salt-, and UV-B radiation-induced stress conditions to analyze both overlapping and divergent gene expression patterns. I decided to write and present this letter because I believe that Li *et al.* [2] provided a synthesis of a premise that's important in scientific research, particularly their stance, and evidence, that peptides are pivotal regulators of plant aging. By doing this, they have placed peptide signaling at the forefront of senescence research, which I support. From the above, it can be gathered that the fundamental theory about leaf senescence sets the stage for practical measures to optimize plant lifespan and performance through intracellular communication. However, charting the spatiotemporal maps of peptide signaling with transcriptional and hormonal networks still requires resolution.

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Research Article

Assessing Groundwater Quality in Islamabad: A Microbiological and Physicochemical Analysis in Compliance with WHO Standards for Potable Water

Sadia Jabeen[1], Niaz Ahmed[1], Muhammad Arshad[2], Abid Ali[3], and Om Parkash[4*]

[1]Department of Microbiology, Wu Lien Teh Institute, Harbin Medical University, Harbin 150081, PR China

[2]Genomics Research Center (Key Laboratory of Gut Microbiota and Pharmacogenomics of Heilongjiang Province), College of Pharmacy, Harbin Medical, University, Harbin, China

[3]Department of Immunology, School of Basic Medical Sciences, Harbin Medical University No. 157, Baojian Road, Nangang District, Harbin City, Heilongjiang Province, 150081, PR China

[4]Department of Pathology, Chandka Medical College, Shaheed Mohtarma Benazir Bhutto Medical University, Larkana, Pakistan

Abstract: The study was conducted in Islamabad, Pakistan, aimed to evaluate the microbiological and physicochemical quality of potable water from various sites. Sampling was conducted in spring 2023 across 20 sites in Islamabad, selection was based on population density and proximity to potential contamination sources (urban runoff, agriculture. Microbiological parameters (total coliforms, fecal coliforms, and *E. coli* using the Most Probable Number (MPN) method and physicochemical parameters such as pH, electrical conductivity (EC), total dissolved solids (TDS), turbidity, hardness, chloride, and nitrate were measured using standard procedures. The findings highlighted significant disparities in *E. coli* contamination across different sites, with the highest count at Site H (21.8) and the absence of *E. coli* at multiple sites (A, E, G, K, and N), indicating a need for site-specific microbial monitoring. Physicochemical analysis revealed variability in water quality, with pH levels ranging from slightly acidic to slightly alkaline. Notably, turbidity levels were highest at Sites D1 measuring a 93.5 Nephelometric Turbidity Unit (NTU), while Site F exhibited the highest EC (4020 µS) and TDS (3015 mg/L). High EC, turbidity and TDS at these specific sites were attributed to geological mineral dissolution, agricultural runoff, and inadequate sewage management. Site H recorded the highest nitrate concentration at 20 mg/L, suggesting contamination from agricultural runoffs or sewage. The study underscores the importance of continuous monitoring and tailored interventions to ensure safe drinking water standards across different locations and mitigate public health risks associated with contaminated groundwater.

Keywords: Groundwater, WHO Standards, Water Quality Monitoring, Coliforms, Physicochemical Parameters, Microbiological Contamination.

1. INTRODUCTION

Water is a fundamental element for all forms of life, serving as an essential component for maintaining human health and well-being globally. Clean drinking water is a basic necessity, utilized for drinking, cooking, personal hygiene, and various domestic activities. Additionally, water plays a crucial role in transportation, hydroelectric power generation, and various industrial and commercial applications. Despite its importance, freshwater, the source of potable water, is a limited resource. Surface freshwater sources (including rivers, lakes, dams, wells, springs, and rain) provide a limited supply of potable water, with only 3% of the world's water being freshwater and an even smaller fraction

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* Corresponding Author: Om Parkash <omparkash.smbbmu@gmail.com>

(0.01%) being suitable for human consumption [1]. On earth the amount of water is now the same as billion years ago, only 2.8% is available as fresh water of which about 20% constitutes groundwater. It is principal freshwater supply which reaches people water demand generally acts for expends demand [2].

Contaminated water poses a significant global health threat. Water pollution causes many diseases like diarrhea, gastroenteritis (infectious diarrhea), stomach cramps and aches and degradation of immune function are included which caused by water pollution [3]. World Health Organization (WHO) evaluated 10% of the global population lacks access to resources for improving drinking water quality [4]. This problem is exacerbated by the increasing contamination of water resources worldwide with microorganisms and chemicals in the 21st century [5]. Each year, 4 billion cases of diarrhea are reported in the world because of the consumption of contaminated water. In Pakistan, rapid population growth and urbanization have further compromised water quality. Several studies across Pakistan including Lahore, Karachi, and Rawalpindi have documented microbial contamination, elevated nitrate levels, and physicochemical imbalances in both surface and groundwater resources [6-8]. Specifically in Islamabad, studies by Haq *et al.* [9] and Shinwari *et al*. [10] reported spatial disparities in groundwater quality, with certain sectors exhibiting fecal coliforms and turbidity beyond WHO guidelines.

Potable water, free from pathogens and harmful chemical substances, is crucial for public health. Various physicochemical parameters, such as microbial contamination, pH, TDS, EC, dissolved oxygen (DO), hardness, and temperature, are used to assess drinking water quality. Physicochemical and microbiological assessments are essential for effective water resource management [11]. Generally, the perception is that if the concentration of any given contaminant is below the standard limit defined according to WHO guidelines, the water is safe but if it is above this limit it is unsafe [12].

Recent studies highlight the alarming escalation of global water pollution, with comprehensive data from 2022 revealing critical regional disparities that demand immediate attention. China leads as the most polluted country globally, accounting for 30% of global pollution, followed by the US at 15%, India at 7%, and Russia at 5%, upsetting water pollution statistics [13]. While 44% of all wastewaters on earth returns to the environment untreated, meaning human waste, household sewage, and toxic medical waste are released directly into ecosystems. A groundbreaking 2024 analysis of 625 studies from 63 countries demonstrated that global urbanization profoundly degraded water quality worldwide, making it the leading landscape change responsible for water-quality deterioration over the past two decades [14]. Furthermore, nitrogen pollution from agriculture and human waste, along with plastics, threatens clean water supplies in many watersheds worldwide, potentially contributing to public health declines. Pollution poses big risks to global clean water supplies, emphasizing the urgent need for comprehensive international water management strategies [15].

Given the critical importance of water quality for public health and well-being, this study aims to assess the quality of potable water consumed in different sectors of Islamabad. This research brings novelty by providing the first comprehensive spatial assessment of groundwater quality across diverse urban and peri-urban areas of Islamabad, establishing baseline data for future monitoring programs. The significance of this study lies in its ability to provide localized insights into drinking water safety, thereby informing both regulatory actions and targeted public health interventions. Specifically, the study aimed to assess the compliance of groundwater quality with established WHO standards, to identify geographic areas exhibiting elevated risks of contamination, and to recommend appropriate remedial measures tailored to the site-specific findings.

2. MATERIALS AND METHODS

2.1. Materials

All chemicals used in the study were of analytical grade with ≥ 99% purity. Sterilized 250 mL borosilicate glass vials (Duran®, Germany) and 1.5 L HDPE plastic containers (Nalgene®, Thermo Fisher Scientific, USA) were used for sample collection. Sterile distilled water was prepared using the Milli-Q® purification system (Millipore®, USA). The pH of water samples was measured using a HI-8424 portable pH meter (Hanna Instruments®,

USA), while EC was recorded using a Model 152 Conductivity Meter (Fisher Scientific®, USA). Turbidity measurements were performed using a DRT-15CE Digital Turbidity Meter (HF Scientific®, USA), calibrated with standard NTU solutions. MacConkey Agar (CM0007, Oxoid®, UK), Peptone Water (Merck®, Germany), and Kovacs' Indole Reagent (Fisher Scientific®, USA) were utilized. Diamond Green Bile Broth (2%) was sourced from Merck®, Germany. Nitrate concentration was determined using the LaMotte® Nitrate Test Kit (Model 3354-01, LaMotte Company®, Maryland, USA) and chloride concentration using the LaMotte® Chloride Test Kit (Model 4503-DR-01, LaMotte Company®, USA). Hardness testing was performed using Reagents 5 and 7 from the LaMotte® Hardness Test Kit.

2.2. Sampling Sites

Sites were designated alphabetically (A–T) and strategically distributed across diverse geographical zones of Islamabad. Samples were collected directly from unfiltered groundwater sources (boreholes/hand pumps) used for drinking. Each site was comprised of five sub-sites, labelled with corresponding alphabetical and numerical identifiers (e.g., A1–A5), resulting in the collection of approximately 100 water samples.

A stratified random sampling design was employed to systematically capture the spatial variability in groundwater quality across twenty different zones of Islamabad to represent a wide range of hydrogeological diversity and contamination risks (sewage infiltration, agricultural runoff) in the region. To ensure statistical significance and minimise local variability, it was decided to collect five samples from each site, giving 100 samples. These sites, identified by abbreviations, are listed as follows: E-7 (A1-A5), F-4 (B1-B5), F-6 (C1-C5), F-7 (D1-D5), F-8 (E1-E5), F-10 (F1-F5), F-11 (G1-G5), G-3 (H1-H5), G-6 (I1-I5), G-7 (J1-J5), G-8 (K1-K5), G-9 (L1-L5), G-10 (M1-M5), G-11 (N1-N5), I-8 (O1-O5), I-9 (P1-P5), I-10 (Q1-Q5), Khanna Pul (R1-R5), Chakshahzad (S1-S5), Bhara kahu (T1-T5) as shown in Figure 1.

This methodological approach ensured comprehensive and detailed coverage of all critical hydrological characteristics of the study area, resulting in highly representative data, eliminating the possibility of systematic errors and providing a sound basis for subsequent integrated analysis.

2.3. Sampling and Preparation for Analysis

Sampling was conducted according to established international standards (ISO 5667-3:2018) for preparing and transporting aqueous samples [16]. It was essential to prevent contamination and secure the samples before delivery to the laboratory. All water samples in this study were collected directly from unfiltered groundwater sources, including boreholes and hand pumps, which are used as primary drinking water sources

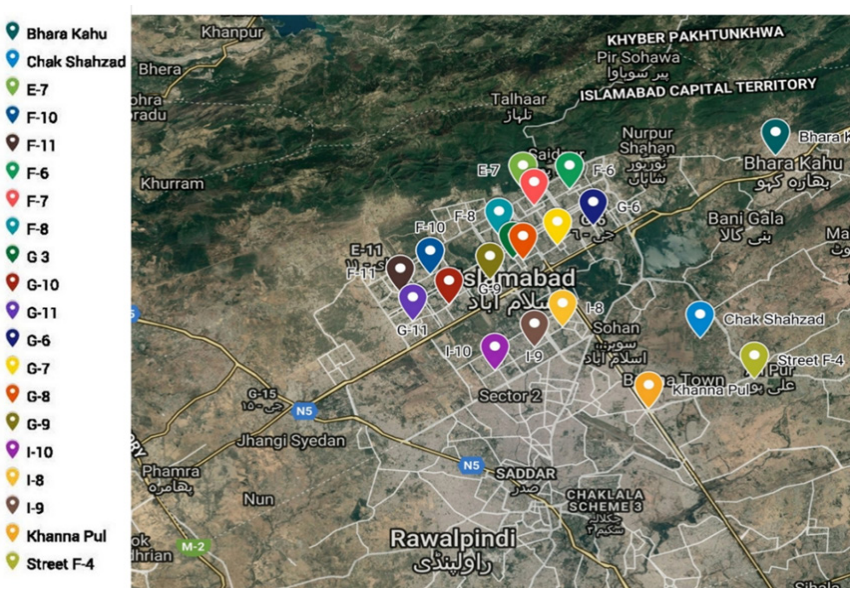

Fig. 1. Various locations of Islamabad selected for testing water quality (source: Google Maps).

by the local residents at all 20 sites. Each sample was collected into sterilised 250 mL borosilicate glass vials, pre-treated and rinsed according to the ISO 19458:2006 protocol [17]. The vials ensured minimal reaction between the walls of the container and the water contained in it as these all vials were composed of high-quality borosilicate glass (Duran®, Germany), known for its chemical inertness and minimal reactivity with aqueous solutions. To analyse physicochemical parameters, an additional 1.5 litres of water was collected in plastic containers, also pretreated with 10% nitric acid and rinsed three times with deionised water to exclude contamination. Sterile distilled water was used as a control. All glassware and instruments were sterilised in an autoclave at 121 °C for 15 minutes prior to field use. Pre-sterilisation was confirmed with autoclave tape indicator.

2.4. Microbiological Analysis

MPN methodology was used for microbiological analysis of water, providing sensitive and reliable detection of coliform bacteria, including fecal coliforms and *E. coli* [18]. MacConkey medium isolates and cultivates coliforms while inhibiting Gram-positive microorganisms. Peptone water is used for the indole test to identify *E. coli*, and Kovacs' indole reagent visualizes indole to confirm the presence of *E. coli*. For MPN estimation, water samples were inoculated into tubes with double concentrations of MacConkey medium and incubated at 35-37 °C for 24 hours. Tubes showing no gas were further incubated to reduce false negatives. Positive samples were then transferred to Diamond Green Bile Broth and incubated at 44 ± 0.5 °C. *E. coli* presence was confirmed by the indole test using Kovacs' reagent, which indicates indole with a red color.

2.5. Physico-Chemical Analysis

Physico-chemical analyses of water samples were carried out using high-precision instrumentation to assess key water quality parameters [19]. EC was measured using a conductivity meter, which had a detection limit of 1 µS/cm, calibrated with standard solutions of 1413 µS/cm and 12.88 mS/cm. Turbidity was measured using a digital turbidity meter, calibrated using standards of 0, 10, 100, and 1000 NTU, and possessing a detection limit of 0.01 NTU. EC was the basis for calculating TDS, calculated by multiplying conductivity readings (in microsiemens, µS or millisiemens, mS) by a factor of 0.75 to obtain accurate dissolved salt concentrations.

The pH was measured using a pH meter, it had a detection limit of 0.01 pH units, and was calibrated using buffer solutions at pH 4.01, 7.01, and 10.01. The presence of sediments was assessed visually, where the water sample was classified as containing sediments ('Present') or not containing sediments ('Absent'). A visual methodology was also used to comprehensively assess the appearance and colour of the water, where the sample was classified as transparent, turbid, reddish, brown turbid or white turbid to provide a comprehensive characterisation of its visual and physicochemical properties.

2.5.1. Hardness as $CaCO_3$

The water hardness of the tested sample was evaluated using the EDTA Titrimetric method for increased precision [20]. The titrimetric method for hardness had a minimum detectable concentration of 0.29 gpg as $CaCO_3$. For this analysis, 12.9 mL of the water sample was taken, and five drops of Hardness Reagent 5 (containing sodium sulfide, sodium hydroxide, and sodium borate) were added. After that, the indicator tablet was added, causing the solution to turn purple. Using the hardness reagent 7 containing EDTA and magnesium chloride the sample was stirred until light blue color without titanium appeared. The hardness was noted in mg/L as $CaCO_3$.

2.5.2. Nitrate-NO_3

Nitrate was determined by LaMotte method which is a nitrate test kit [21]. For chemical analysis, nitrate and chloride detection limits were derived from the LaMotte test kit documentation: 0.1 mg/L for nitrate. From the sample a volume of 2.5 mL of water was taken and mixed with 5 mL of a mixed acid reagent which contains acetic acid, copper sulfate, ammonium chloride, sodium chloride, citric acid and sodium phosphate. Nitrate reducing powder was then added to the solution and after shaking, visually the solution was tested against a color standard using a Nitrate – N comparator. Nitrate content is expressed in ppm, this way aiming to give the level of nitrate in the freshwater.

2.5.3. Chloride

The LaMotte automatic burette chloride test kit was also employed for the determination of chloride levels [22]. According to the test Kit manual, the detection limit for chloride analysis was established at 0.5 mg/L. A 50 mL water sample was taken in an Erlenmeyer flask, and chloride reagents were added dropwise. If no red color appeared instantly in the solution, then sodium hydroxide was added in drops till a red color developed. Thereafter, the treatment with hydrogen peroxide and sulfuric acid was performed, after which the solution was titrated with silver nitrate until the color changed from yellow to orange, brown. When using the formula, ppm chloride = 0.1 x (burette reading-0.2); the estimation of chloride concentration was performed by subtracting the mL on the burette from the value obtained by placing the internal standards in the whole sample drawn out.

3. RESULTS

The study aimed to evaluate the bore water's quality by examining various crucial characteristics, including appearance, pH, EC, TDS, chloride, hardness, nitrate, turbidity, total coliforms, fecal coliforms, and *E. coli* count. As shown in Figure 2, all of the samples collected from site P and R were found to be clear in appearance. Visual analysis of water from site A, B, C, E, F, G, I, J, K, L, M, Q, and S were determined to be clear, with the exception of one sub-site which exhibited turbidity. Among the samples collected from site H, N, O, and T, two of the sub-sites were found to be turbid, while the other three were clear. Out of the samples collected from site D, three sub-sites exhibited higher levels of turbidity, while the rest of the samples had a clear look. These findings indicate that the majority of water samples from all sites were visually clear, varying levels of turbidity were observed at a minority of locations, most notably at sites D, H, N, O, and T.

Sterile distilled water was processed alongside all experimental samples demonstrated no contamination, thereby confirming assay specificity. Among the tested chemical parameters, the pH values at different locations varied from mildly acidic to mildly alkaline. The majority of sites maintained a pH level that fell within the permitted range for drinking water, which is typically between 6.5 and 8.5 (Table 1), indicating that most locations falling within the acceptable drinking water standards of 6.5-8.5 pH units.

The turbidity levels differed greatly among the sites, with some locations having clear water and others displaying considerable turbidity. Site A, specifically sub-site D1, had the highest turbidity with a measurement of 93.5 NTU, indicating a significant presence of suspended particles. Additional noteworthy high turbidity measurements are seen at sub-sites H4 (42.5 NTU), G5 (23.8 NTU), and N4 (58.8 NTU) (Table 1). These findings indicate that the water quality is not uniform across the area and that specific locations are significantly impacted by pollution or environmental disturbances.

The EC values, which represent the overall number of ions in the water, varied significantly from 265 µS to 4020 µS. Locations with the highest EC values, such as Site F (reaching up to 4020

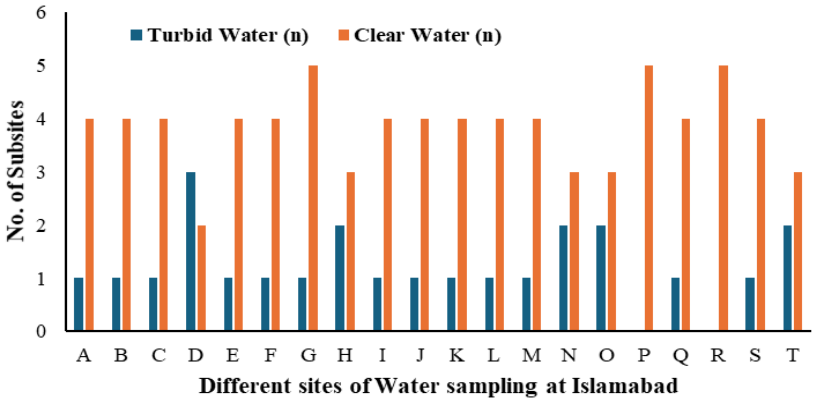

Fig. 2. Findings of physical appearance of water at various sub-sites of water sampling.

µS), indicate a notable concentration of dissolved salts, potentially resulting from the dissolution of minerals or human activities. In contrast, locations such as Site I, which have lower EC values (265 to 695 µS) (Table 1), indicate lesser ionic content and potential dilution effects.

The TDS levels varied between 198 mg/L to 3015 mg/L at the different sites. Elevated TDS levels observed at Site F (reaching a maximum of 3015 mg/L) and Site D (reaching a maximum of 1402 mg/L) indicate substantial mineral content, which may be attributable to either the geological characteristics of the area or potential pollution sources. The lower TDS values seen at sites such as Site I (from 198 to 524 mg/L) indicate the presence of relatively purer water (Table 1).

The nitrate levels observed at most sites were predominantly low, with a range of values between 0.25 mg/L and 20 mg/L. Site H shows the highest nitrate concentration at 20 mg/L, indicating the presence of agricultural runoff or sewage contamination. The nitrate levels observed at sites, including Site Q, generally remained low, with values from 0.25 to 1 mg/L (Table 1).

Table 1. Findings of physicochemical investigation of water samples and their compliance with WHO standards.

Parameter	Observed Range	Mean value	Standard Deviation	Key Findings	WHO Standards	Compliance Status
pH	6.5 – 8.0	7.0	± 0.4	- Majority within WHO range (6.5–8.5). - Highest at Site D (7.4–8.0). - Lowest at Site B (6.5–7.5).	6.5 – 8.5	Compliant (All sites)
Turbidity (NTU)	0 – 93.5	12	± 9.2	- Exceeded at sub-sites D1 (93.5), H4 (42.5), G5 (23.8), N4 (58.8). - Most sub-sites <5.	< 5	Non-compliant (exceeded at D1, H4, G5, N4)
EC (µS/cm)	265 – 4020	312	± 80	- Highest at Site F (4020 µS/cm). - Lowest at Site I (265–695 µS/cm). - Indicates high mineralization.	< 400	Non-compliant (exceeded at Sites F, D, etc.)
TDS (mg/L)	198 – 3015	223	± 69	- Highest at Site F (3015 mg/L). - Lowest at Site I (198–524 mg/L). - Exceeds WHO limit at Sites F, D.	< 1000	Non-compliant (exceeded at Sites F, D)
Nitrate (NO_3, mg/L)	0.25 – 20	08	± 4	- Highest at Site H (20 mg/L). - Lower values at Sites Q (0.25–1 mg/L).	< 10	Non-compliant (exceeded at Site H)
Hardness (gpg)	10.5 to > 10.5	10	± 2	- Water at Sites B5, J, and K was classified as very hard, with hardness levels >10.5 gpg. Site I exhibited both hard and very hard water, with values ranging from 10.5 gpg to >10.5 gpg.	< 1	Non-compliant (exceeded at Sites B, J, K, I)
Chloride (mg/L)	9.8 – 39.4	10.1	± 3	- Highest at Site F (39.4 mg/L). - Lowest at Site S (9.8 mg/L).	< 250	Compliant (All sites)

Investigation of water hardness revealed that the water in the study area was very hard in most cases. Water samples from Sites B5, J, and K consistently exhibited very hard characteristics, exceeding 10.5 gpg. Similarly, water at Site I ranged from 10.5 gpg to values exceeding 10.5 gpg, classifying it as hard to very hard.

The examination of chloride concentrations in the water samples unveiled significant variations among the locations. At site F, the chloride level reached a maximum of 39.4 mg/L. In contrast, site S had the lowest chloride content, measuring 9.8 mg/L. The results demonstrate the variation in chloride concentration among different places, (Table 1). The microbiological analysis of groundwater quality in the study area unveiled alarming levels of contamination, raising serious concerns about public health and water safety. Total Coliforms were detected in every sampling site, with concentrations ranging from 8.4 to a staggering 36.8 CFU/100 mL (Table 2). The highest counts were found at Sites H (36.8 CFU/100 mL), B (32.2 CFU/100 mL), and Q (32.6 CFU/100 mL), indicating significant pollution. In contrast, the lowest levels were recorded at Sites O (8.4 CFU/100 mL), N (11 CFU/100 mL), and K (11.8 CFU/100 mL). These findings indicate that all groundwater sources in the study area are microbiologically contaminated with Total Coliforms, exceeding WHO safety standards and posing a significant threat to public health.

When it comes to Fecal Coliforms, the situation is equally concerning. These bacteria, which indicate fecal contamination, ranged from 0.4 to 25.6 CFU/100 mL. The highest levels were again at Sites H (25.6 CFU/100 mL) and B (22 CFU/100 mL), while Sites E (0.9 CFU/100 mL), I (2.6 CFU/100 mL), and N (0.4 CFU/100 mL) showed relatively lower counts. However, only Sites E, I, and N managed to comply with the WHO standard of 0 CFU/100 mL, underscoring a critical need for improved sanitation measures (Table 2). These findings indicate widespread fecal contamination across most sites, with only a few meetings the WHO standard, highlighting an urgent need for improved sanitation and water treatment measures.

The presence of *E. coli* further compounds the issue, with concentrations ranging from 0 to 21.8 CFU/100 mL, peaking at Site H (21.8 CFU/100 mL) (Table 2). While some sites like A, E, G, K, and N showed no detectable *E. coli*, others such as B, C, D, and F did not fare as well, contributing to a non-compliance status against the WHO standard (Table 2). These findings indicate that *E. coli* contamination is present in several groundwater sources, with some sites exceeding safe limits, further emphasizing the health risks and the need for urgent water quality interventions.

4. DISCUSSION

The comprehensive evaluation of groundwater quality in Islamabad reveals significant spatial heterogeneity in both physicochemical and microbiological parameters, with important implications for public health and water

Table 2. Microbiological contamination levels in groundwater and their compliance with WHO standards.

Parameter	Observed Range (CFU/100 mL)	Key Findings	WHO Standards	Compliance Status
Total Coliforms	8.4 - 36.8	- Highest at Sites B (32.2), H (36.8), Q (32.6). - Lowest at Sites K (11.8), N (11), O (8.4).	0 CFU/100 mL	Non-compliant (presence detected at all sites)
Fecal Coliforms	0.4 - 25.6	- Highest at Sites B (22), H (25.6). - Lowest at Sites E (0.9), I (2.6), N (0.4).	0 CFU/100 mL	Non-compliant (presence detected at all sites except E, I, N)
E. coli	0 - 21.8	- Highest at Site H (21.8 CFU/100 mL). - Absent at Sites A, E, G, K, N.	0 CFU/100 mL	Non-compliant (detected at Sites B, C, D, F, H, etc.)

management. The pH values recorded across all sampling sites (6.5-8.0) remained within the WHO acceptable range of 6.5-8.5 for drinking water, indicating generally satisfactory acidic-alkaline balance. This finding aligns with previous groundwater quality assessments conducted in similar hydrogeological settings. Khan *et al.* [23] reported comparable pH ranges (6.4-8.2) in their comprehensive study of groundwater quality in South Asian aquifers, suggesting that the natural buffering capacity of the aquifer system maintains pH stability. The slight variations observed between sites (highest at Site D: 7.4-8.0; lowest at Site B: 6.5-7.5) likely reflect localized differences in rock-water interactions and mineral dissolution processes, consistent with findings reported by Adimalla and Qian [24] in their global review of groundwater geochemistry.

The turbidity measurements revealed significant spatial heterogeneity, with values ranging from 0 to 93.5 NTU. Four sub-sites (D1, H4, G5, N4) exceeded the WHO guideline of < 5 NTU, with sub-site D1 showing critically high turbidity (93.5 NTU). These elevated turbidity levels indicate substantial suspended particulate matter, potentially originating from surface infiltration, aquifer disturbance, or inadequate wellhead protection. Similar findings were reported by Khatri and Tyagi [25] in their assessment of groundwater quality in agricultural regions, where turbidity spikes were attributed to poor well construction and surface contamination. The high turbidity at specific locations suggests localized contamination sources that require immediate remedial attention.

The EC values exhibited extreme variation (265-4020 µS/cm), with Site F showing exceptionally high mineralization (4020 µS/cm). This substantial range indicates significant hydrogeochemical diversity across the study area. The strong positive correlation between EC and TDS ($R^2 > 0.95$) confirms the reliability of conductivity as a proxy for dissolved salt content, consistent with established hydrogeochemical principles [26]. The elevated EC and TDS values at Sites F and D (TDS: 3015 mg/L and 1402 mg/L, respectively) exceed WHO guidelines (< 1000 mg/L), indicating potential salinization processes. Such high mineralization patterns have been documented in similar hydrogeological settings by Mohit and Suprita [26], who attributed elevated TDS to prolonged water-rock interactions and anthropogenic contamination.

Nitrate levels varied considerably (0.25-20 mg/L), with Site H exhibiting concentrations (20 mg/L) that exceed WHO standards (< 10 mg/L). Elevated nitrate concentrations in groundwater are typically indicative of agricultural runoff, sewage infiltration, or septic system leakage. The spatial distribution of nitrate contamination, with highest levels at Site H and lowest at Site Q (0.25-1 mg/L), suggests point-source contamination rather than diffuse pollution. This pattern is consistent with findings reported by Liu *et al.* [27] in their study of groundwater contamination in rural areas, where localized nitrate hotspots were linked to intensive agricultural practices and inadequate waste management.

Study on water hardness demonstrated that water hardness is a significant concern across the study area, with most sites falling into the very hard category. The consistently high hardness levels at Sites B5, J, and K point to persistent exposure to elevated concentrations of calcium and magnesium ions. Site I, showing a range from hard to very hard, further supports this trend. The widespread distribution of such hardness levels suggests underlying geological formations rich in mineral deposits or prolonged interaction with mineral-laden groundwater. This finding is consistent with studies by Zhang *et al.* [28], who reported similar hardness patterns in carbonate aquifer systems.

Chloride concentrations remained well within WHO guidelines (<250 mg/L), ranging from 9.8 to 39.4 mg/L. The relatively low chloride levels across all sites suggest minimal influence from marine intrusion or industrial contamination, which is consistent with the inland location of the study area. Similar chloride patterns have been reported by Panjwani *et al.* [29] in their assessment of groundwater quality in crystalline aquifers.

The universal presence of total coliforms across all sampling sites (8.4-36.8 CFU/100 mL) represents a critical public health concern, as WHO standards mandate zero coliform presence in drinking water. The highest contamination levels at Sites H (36.8 CFU/100 mL), B (32.2 CFU/100 mL), and Q (32.6 CFU/100 mL) indicate severe bacterial pollution. These findings are consistent

with previous studies in similar settings, where Traoré *et al.* [30] reported widespread coliform contamination in groundwater sources from developing regions, attributing the contamination to inadequate sanitation infrastructure and poor wellhead protection.

The detection of fecal coliforms (0.4-25.6 CFU/100 mL) and *E. coli* (0-21.8 CFU/100 mL) across multiple sites indicates direct fecal contamination, posing serious health risks. The co-occurrence of high levels at Sites H and B suggests common contamination sources, potentially linked to sewage infiltration or animal waste runoff. Only three sites (E, I, and N) showed compliance with WHO standards for fecal coliforms, while several sites remained *E. coli*-free. The presence of these indicators strongly suggests inadequate source protection and contamination pathways from surface activities, consistent with findings reported by Bekoe *et al.* [31] in studies of groundwater vulnerability in populated areas.

The comprehensive analysis reveals a complex groundwater quality scenario characterized by significant spatial heterogeneity in both physicochemical and microbiological parameters. The simultaneous occurrence of elevated TDS, hardness, and microbial contamination at certain sites suggests multiple contamination sources and inadequate source protection measures. This multi-parameter contamination pattern has been documented in similar hydrogeological settings by Zehra *et al.* [32], who emphasized the need for integrated water quality management approaches.

The microbiological contamination presents the most immediate health threat, as consumption of bacteria-contaminated water can lead to waterborne diseases including gastroenteritis, typhoid, and cholera [33]. The physicochemical non-compliance, particularly elevated TDS and hardness, poses long-term health risks and aesthetic concerns that may affect water acceptability and consumption patterns.

In addition to WHO guidelines, Pakistan's National Drinking Water Quality Standards (NDWQS) were also considered for a more regionally contextualized evaluation. The NDWQS, formulated by the Pakistan Council of Research in Water Resources (PCRWR), prescribe permissible limits for various water quality parameters including pH (6.5-8.5), turbidity (< 5 NTU), EC (< 1,500 µS/cm), TDS (< 1,000 mg/L), nitrate (< 10 mg/L as NO_3^-), hardness (< 500 mg/L as $CaCO_3$), and chloride (< 250 mg/L). When the observed data were cross compared with both WHO and NDWQS guidelines, the compliance status remained largely consistent, except for EC, where the NDWQS upper limit is slightly higher than WHO's reference for potable water quality [34].

Based on these findings, immediate implementation of water treatment systems, improved source protection measures, and regular monitoring protocols are essential. The spatial variability in contamination patterns suggests that site-specific treatment approaches may be more effective than uniform interventions, consistent with recommendations by Bekoe *et al.* [31] for groundwater quality management in heterogeneous aquifer systems.

This study's scope was limited to a subset of key water quality indicators, including pH, turbidity, EC, TDS, nitrate, hardness, and chloride, rather than the full range recommended by standards. These parameters were chosen for their public health relevance and the feasibility of their measurement with available resources. Consequently, contaminants like heavy metals and pesticides were excluded from the analysis. The primary reason for this exclusion was the lack of access to advanced analytical equipment. Despite these constraints, the selected indicators provide a reliable assessment of groundwater suitability for consumption in the area. Future research should aim to incorporate a more comprehensive set of parameters for a complete risk assessment.

4. CONCLUSIONS

This study aimed to assess the physicochemical and microbiological quality of groundwater used for drinking purposes across various sectors of Islamabad, comparing findings against WHO standards. The assessment of groundwater quality revealed variability in water quality. The pH and chloride levels were in limits across most sites. However, various physicochemical parameters like EC, turbidity, TDS, nitrate, hardness, and chloride, frequently exceeded the permissible WHO limits in specific locations (e.g., Sites D1, H4, G5, N4 for

turbidity; Sites F, D for EC/TDS; Site H for nitrate; Sites B5, J, K for hardness), suggesting issues related to mineralization, potential pollution from agricultural or urban runoff, and geological factors. Microbiological analysis revealed widespread contamination, with total coliforms, fecal coliforms, and E. coli. Total Coliforms were present in all tested samples, indicating a universal failure to meet the WHO standard of zero coliforms per 100 mL. Furthermore, the frequent detection of Fecal Coliforms and *E. coli* (particularly high at sites like H and B) points towards significant fecal contamination in many areas. The presence of these pathogens poses significant health risks, emphasizing the need for immediate remedial actions. Overall, the groundwater quality in many parts of Islamabad is compromised and often unsuitable for direct consumption without treatment, based on WHO standards. To address Islamabad's groundwater contamination, immediate actions should include installing filtration systems (e.g., RO) in high-turbidity areas and nitrate removal units in agricultural zones, along with repairing sewage infrastructure near highly contaminated sites.

5. CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Research Article

Evaluation of Selected Synthetic and Botanical Insecticides against *Tuta absoluta* (Lepidoptera: Gelechiidae) under Field Conditions

Ghulam Qader Mangrio[1], Arfan Ahmed Gilal[1*], Lubna Bashir Rajput[1], Jamal-U-Ddin Hajano[2], Zakia Panhwar[3], Muhammad Ibrahim Kubar[1], Naimatullah Koondhar[4], and Abdul Hayee Gabol[1]

[1]Department of Entomology, Faculty of Crop Protection, Sindh Agriculture University, Tandojam, Pakistan
[2]Department of Plant Pathology, Faculty of Crop Protection, Sindh Agriculture University, Tandojam, Pakistan
[3]Southern Zone, Agricultural Research Center, Pakistan Agricultural Research Council, Karachi, Pakistan
[4]Department of Plant Pathology, Faculty of Agriculture, Lasbela University of Agriculture, Water and Marine Sciences, Lasbela, Pakistan

Abstract: The invasive tomato leafminer *Tuta absoluta* has recently caused heavy losses to tomato growing areas around the world including Pakistan. Considering its significant damage potential, it is essential to conduct experiments using locally available botanicals and synthetic insecticides. Therefore, studies were conducted at a farmer's field in the district Shaheed Benazir Abad, Sindh, Pakistan during complete tomato season of 2023-24. Synthetic insecticides, i.e., Tracer 450SC ® (Spinosad 480 g/L), Belt 480 SC ® (Flubendiamide 480 g/L), Novastar 56EC ® (Bifenthrin 5%+ Abamectin 0.6%), Talstar 10EC ® (Bifenthrin), and Trigard 750 g/kg ® (Cyromazine) and botanical insecticides, i.e., Neem (*Azadirachta indica* A. Juss.), Tobacco (*Nicotiana tabacum* L.), Datura, (*Datura stramonium* L.), Peppermint (*Mentha piperita* L.), and Eucalyptus (*Eucalyptus camaldulensis* Dehn.), along with untreated control were evaluated against *T. absoluta* During both sprays, synthetic insecticides were found to be comparatively more effective than botanicals, with Flubendiamide and Spinosad being the two most effective insecticides. The effectiveness of all insecticides was comparatively better in the second spray than first spray. After one week of the two sprays, the maximum infestation reduction on tomato leaves and fruits was recorded with Flubendiamide (73.34% and 85.60%, respectively), followed by Spinosad (70.58% and 83.57%, respectively), whereas Eucalyptus and Datura were the least effective insecticides. Among botanicals, Neem resulted in a maximum corrected reduction in *T. absoluta* infestation on tomato leaves (52.33%) and fruits (55.20%). Due to the effectiveness of insecticides in reducing *T. absoluta* infestation, a significant effect on tomato fruit yield was also observed as maximum yield was recorded with Flubendiamide (433.40 ± 5.46 maunds per acre) and Spinosad (420.80 ± 3.20 maunds per acre) treatments, whereas neem (389.60 ± 4.86 maunds per acre) gave maximum yield among botanicals. Therefore, it is recommended that due to the better performance of Flubendiamide and Spinosad, they should be included in the integrated management of *T. absoluta* infestation in tomatoes, whereas Neem can also be used as botanical insecticide due to its effectiveness against it.

Keywords: Botanical insecticides, Infestation, Invasive, Management, Synthetic Insecticides, Tomato, *Tuta absoluta*.

1. INTRODUCTION

Tuta absoluta, commonly known as tomato leafminer, has been identified as one of the most destructive pests of tomatoes which can cause 80 to 100% yield losses when the conditions favor its growth and development [1-3]. Therefore, having such a huge damage potential, *T. absoluta* require early detection, monitoring, and control measures. Pheromone and various colored light traps, especially golden and blue, were found effective particularly in the monitoring of *T. absoluta*. Other

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* Corresponding Author: Arfan Ahmed Gilal <aagilal@sau.edu.pk>

studies also highlight the importance of pheromone and light traps in monitoring and mass destruction of *T. absoluta*, hence are included as key components in its integrated management [4-8].

Although various management tools are available for the control of *T. absoluta*, synthetic insecticides are still the most dependable and widely used approach to managing *T. absoluta* under field and greenhouse tomatoes [9]. However, it has been observed that most of the pesticides are unable to get desired control of *T. absoluta* because of its cryptic nature of damage [9-11] along with potential to develop resistance against most of the widely used insecticides [12-18]. Moreover, wide scale and frequent use of insecticides pose great threats to non-targeted organisms, humans, and the environment [19, 20]. Accordingly, it creates potential for the botanical and biorational pesticides to be used against *T. absoluta* because of their specific mode of action and less hazardous to non-target organisms and relatively safe to the environment [21].

Therefore, studies on the effectiveness of various botanicals against *T. absoluta* have been conducted in various countries which provided promising results to manage its various life stages [22-26]. Among botanicals, *A. indica* ethanolic extract and petroleum ether extract of *J. curcus* seeds were evaluated on eggs and larval stages of *T. absoluta* as both the extracts cause its significant mortality [23, 24, 27]. *Simmondsia chinsis* seed extracts applied at 100 percent concentration resulted in 75 percent mortality on 2nd instar larvae of *T. absoluta* [28]. It has also been observed that ethanolic leaf extract obtained from *P. amalago* resulted in 70% mortality of larvae and pupae within two-days exposure time, thus exhibiting acute toxicity at the concentration of 2,000 mg/L [29]. Moreover, the aqueous extracts of *M. azedarach, P. zonale, A. sativum, A. cepa, O. basilicum* also exhibited moderate to high mortality of different stages of *T. absoluta* [30]. Furthermore, crude extracts of three plants, i.e., *A. indica* seed, *C. citrates*, and *A. sativum* also resulted in 98, 97, and 95 % mortality of *T. absoluta* larvae, respectively within 7 days of the exposure in Ethiopia [25].

Recently, the presence of *T. absoluta* has been reported from tomato growing areas of Punjab, Khyber Pakhtunkhwa, and Sindh provinces of Pakistan [31-33], therefore, it has become imminent to conduct studies on its management using less toxic, new chemistry synthetic insecticides along with locally available botanicals against it. The results obtained could be helpful to determine the most effective synthetic and botanical insecticides that can be included in the integrated management of *T. absoluta* to restrict its losses and further spread in the country.

2. MATERIALS AND METHODS

The botanicals were obtained from the study areas, whereas synthetic insecticides were purchased from the authorized dealers. Two sprays were performed in the study as data were taken from ten randomly selected tomato plants per replications to count total and infested leaves and stems to calculate infestation percentage.

2.1. Study Location and Cultivation of Tomato

The study was conducted during the tomato growing season of 2023-24 at a farmer's field located in the district of Shaheed Benazir Abad, Sindh, Pakistan. Tomato variety Desi Local was cultivated at its recommended dose of 150 grams per acre. All the agronomic practices were applied as per the recommendations. The size of individual replication was maintained at 100 ft^2.

2.2. Treatments

The following synthetic and botanical insecticides were used in the study:

Tracer 450SC® (Spinosad 480 g/L), Corteva Agriscience @ 150 mililiter per acre
Belt 480 SC® (Flubendiamide 480 g/L), Bayer Crop Science @ 30 mililiter per acre
Novastar 56EC® (Bifenthrin 5%+ Abamectin 0.6%), FMC, Pakistan @ 500 mililiter per acre
Talstar 10EC® (Bifenthrin), FMC, Pakistan @ 400ml per acre
Trigard 750 g/kg® (Cyromazine), Syngenta Pakistan Ltd. @ 150 gram/acre
Neem, *Azadirachta indica* A. Juss.
Tobacco, *Nicotiana tabacum* L.
Datura, *Datura stramonium* L.
Peppermint, *Mentha piperita* L.
Eucalyptus, *Eucalyptus camaldulensis* Dehn.
Control (only water)

2.3. Preparation of Stock Solution of Botanical Pesticides

All the above-mentioned plant materials were collected afresh from the surroundings of the study area and brought in laboratory of Entomology Department, Sindh Agriculture University, Tando Jam, Pakistan. The procedure for preparation of botanicals was adopted from Kunbhar *et al*. [34] with slight modification. Five kilograms of each plant material was washed with distilled water and kept for air drying in the laboratory. Afterwards, plant materials were grinded using electric blender (GEEPAS China, Model GCG289) to get their small pieces and then boiled in ten litters of water for 30 minutes. The boiled material was then placed under shade for cooling and sieved from muslin cloth to get the fine stock solution for the spray. In the final stock solution, 125-gram detergent powder was added to avoid clotting of the planting materials and improve their dispersing quality. The calibration of water and individual botanical extract was done as per recommendation before their application.

2.4. Application of Insecticides, Experimental Design, Data Collection, and Analysis

Considering population development, the spray schedule against *T. absoluta* was designed as two sprays were applied during the study keeping in view the economic threshold levels i.e., 25-30% leaf or 10-12% fruit infestation. All the insecticides were applied as per their recommended doses (given in section 2.2). Experiment was arranged in a randomized complete block design with five replications maintained for the individual insecticide treatment.

Data on the infestation of *T. absoluta* on tomatoes was collected before the sprays, whereas subsequent observations were taken after 24, 48, 72, and 96 hours, and one week after the application of various insecticides. Moreover, ten randomly selected tomato plants per replication were observed to record *T. absoluta* infestation. *Tuta absoluta* infestation was assessed by counting the number of healthy and infested leaves and fruits by observing its characteristic damage symptoms, i.e., distinct 'blotch' mines with an accumulation of dark-colored frass in mines of leaves and holes in fruits.

The corrected reduction percentage in the *T. absoluta* infestations due to the application of various insecticides was calculated using Henderson and Tilton formula [35] given below:

$$Corrected\ \% = \left(1 - \frac{n\ in\ Co\ before\ treatment * n\ in\ T\ after\ treatment}{n\ in\ Co\ after\ treatment * n\ in\ T\ before\ tretment}\right) \times 100$$

n = number or infestation, Co = control, T = treatment

The Analysis of Variance along with the Least Significant Difference (LSD) test at 5% probability was used for the data analysis using STATISTIX 8.1 computer software.

3. RESULTS

3.1. Performance of Various Insecticides to Manage Infestation of *Tuta absoluta* on Tomato Leaves after First Spray

Figure 1 illustrates the results regarding the performance of the first spray of various botanical and synthetic insecticides on the infestation of *T. absoluta* on tomato leaves at various observation intervals. A non-significant ($F = 0.18$, $P = 0.9973$) difference was observed in the mean infestation of *T. absoluta* on tomato leaves of various treatment insecticides before spray as the infestation ranges between 28.21 ± 1.98 to 30.87 ± 2.63% among the treatments. Although an increasing trend in the infestation of *T. absoluta* on tomato leaves was recorded in the control, a reduction in its infestation was recorded in various insecticide treatments at various intervals of their application. Thus, after 24-hours after the application of insecticides, significantly ($F = 2.34$, $P = 0.0114$) the lowest infestation (21.03 ± 1.74%) on tomato leaves was recorded with Flubendiamide, followed by Spinosad (22.13 ± 1.84%) and Bifenthrin (24.90 ± 1.93%), whereas control showed the highest (31.23 ± 3.25%) infestation, followed by Eucalyptus (28.00 ± 1.75%) and Datura (27.70 ± 1.76%) treatments. Afterwards, a further decline in the infestation of *T. absoluta* was observed in insecticide treatments, but they exhibited significant differences in their effectiveness at various observation intervals i.e., 48-hours ($F = 3.45$, $P = 0.003$), 72-hours ($F = 4.79$, $P < 0.001$), 96-hours ($F = 5.63$, $P < 0.001$), and one week ($F = 9.25$, $P < 0.001$) of the insecticide application. Therefore, at the end of one week after the application of various treatments, the lowest

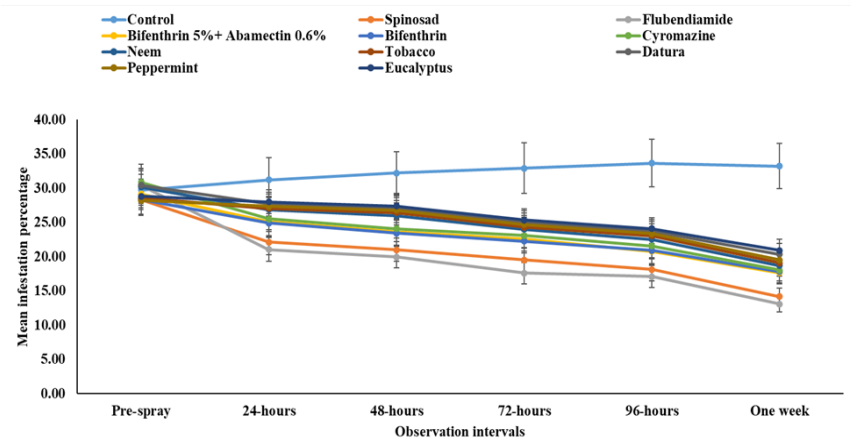

Fig. 1. Impact of various synthetic and botanical insecticides on the mean infestation percentage of *Tuta absoluta* on tomato leaves after the 1st spray.

infestation (13.07 ± 1.14%) of *T. absoluta* on tomato leaves was recorded with Flubendiamide, followed by 14.17 ± 1.27, 17.63 ± 1.60, and 17.80 ± 1.62% infestation recorded on Spinosad, Bifenthrin + Abamectin, and Bifenthrin insecticides. Among insecticide treatments, the highest (20.90 ± 1.62%) *T. absoluta* infestation on leaves was observed with Eucalyptus, followed by Datura (20.37 ± 1.57%) and Peppermint (19.50 ± 1.55%) treatments. Among botanicals, Neem (18.70 ± 1.56%) and Tobacco (19.10 ± 1.51%) were found comparable with some of the synthetic insecticides i.e., Cyromazine, Bifenthrin, and Bifenthrin + Abamectin in the reduction of *T. absoluta* infestation on tomato leaves. Overall, the highest *T. absoluta* infestation on tomato leaves was recorded in control at various observation intervals.

The corrected percent reduction in the mean infestation of *T. absoluta* on tomato leaves due to the first application of various insecticide treatments using Henderson and Tilton formula [35] is given in Figure 2. It was observed that at the end of one week after the application of various synthetic and botanical insecticides, none of the insecticide was capable of reducing 100% infestation of *T. absoluta* on tomato leaves. However, synthetic insecticides were comparatively more effective than botanicals with Flubendiamide and Spinosad being found to be the two significantly effective insecticides. Results also indicate that reduction in the *T. absoluta* infestation was observed after 24-hours of the application of various insecticide as maximum corrected infestation reduction (32.66%) was recorded with Flubendiamide, followed by Spinosad (29.14%) and Bifenthrin (20.285%). Among botanicals, the maximum infestation reduction was observed with Neem (13.98%) and Tobacco (13.45%). Overall, the minimum infestation reduction of *T. absoluta* was recorded

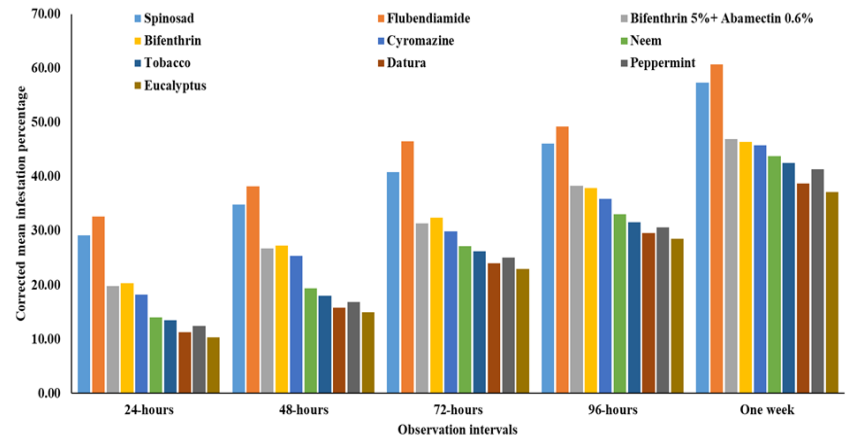

Fig. 2. Impact of various synthetic and botanical insecticides on the corrected mean infestation reduction percentage of *Tuta absoluta* on tomato leaves after 1st spray.

with Eucalyptus (10.35%) and Datura (11.31%). The corrected mean infestation of *T. absoluta* on tomato leaves in all the insecticide treatments showed a further decline till the end of one week of their application, especially in synthetic insecticides. Accordingly, the Flubendiamide (60.68%) and Spinosad (57.37%) insecticides were found most effective to reduce *T. absoluta* infestation on tomato leaves, whereas Eucalyptus (37.11%) and Datura (38.72%) were found the least effective to control the infestation of *T. absoluta.* Among botanicals, the performance of Neem (43.73%) and Tobacco (42.53%) was not significantly different from Bifenthrin + Abamectin (46.94%), Bifenthrin (46.44%), and Cyromazine (45.74%) to reduce *T. absoluta* infestation on tomato leaves.

3.2. Performance of Various Insecticides to Manage Infestation of *Tuta absoluta* on Tomato Leaves after Second Spray

Results regarding the impact of the second spray of various synthetic and botanical insecticides on the mean infestation of *T. absoluta* on tomato leaves at various time intervals are given in Figure 3. Comparatively lower level of *T. absoluta* infestation on tomato leaves was recorded in all the insecticide treatments, however, the application of various insecticides was found effective to significantly reduce the infestation, especially synthetic insecticides. The mean *T. absoluta* infestation in various treatments were not significantly ($F = 0.03$, $P = 1.000$) from each other, which ranged between 24.24 ± 2.44 to 25.60 ± 2.56%. After 24-hours of the application of various insecticides, a reduction was recorded in the mean infestation of *T. absoluta* on tomato leaves with the lowest infestation observed with Flubendiamide (16.60 ± 1.91%) and Spinosad (17.83 ± 2.23%), followed by Bifenthrin + Abamectin (18.37 ± 2.59%) and Bifenthrin (18.70 ± 2.10%). Among treatments, the maximum infestation (25.97 ± 2.31%) was recorded in control, followed by Eucalyptus (21.50 ± 1.99%), Datura (21.17 ± 2.01%), and Peppermint (20.37 ± 2.05%). Overall, a non-significant ($F = 1.67$, $P = 0.0866$) difference in the performance of different insecticides was recorded after 24-hours after their application to reduce *T. absoluta* infestation on tomato leaves. The effectiveness of various insecticides to reduce *T. absoluta* infestation on leaves was recorded till the end of one week of their application, however, a significant difference in their effectiveness was observed at various observation intervals i.e., 48-hours ($F = 2.92$, $P = 0.0016$), 72-hours ($F = 5.62$, $P < 0.001$), 96-hours ($F = 7.72$, $P < 0.001$), and one week ($F = 15.32$, $P < 0.001$). Thus, one week after the application of various insecticides against *T. absoluta,* significantly the lowest infestation (8.03 ± 1.09%) was recorded with Flubendiamide that was not significantly different from infestation observed with Spinosad (8.87 ± 1.21%). Among insecticide treatments, the highest *T. absoluta* infestation on leaves was recorded with Eucalyptus (13.97 ± 1.57%) and Datura (13.70 ± 1.59%), followed by Tobacco (13.43 ± 1.43%) and Peppermint (13.07 ± 1.35%), whereas Neem was found to be most effective botanical with lowest infestation (12.53 ± 1.43%) on tomato leaves. Overall, the highest *T. absoluta* infestation on tomato leaves was recorded in control at various observation intervals.

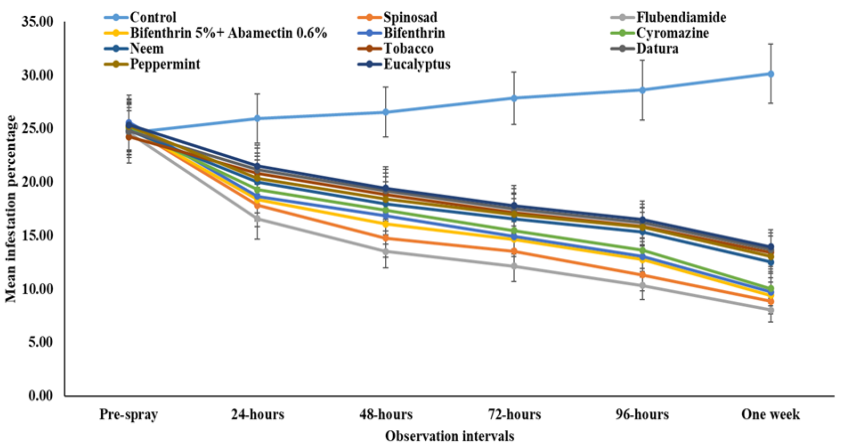

Fig. 3. Impact of various synthetic and botanical insecticides on the mean infestation percentage of *Tuta absoluta* on tomato leaves after 2nd spray.

The corrected infestation reduction of *T. absoluta* on tomato leaves due to the application of various insecticides is given in Figure 4. Like 1st spray, Flubendiamide and Spinosad were found to be the most effective insecticides to reduce *T. absoluta* infestation on leaves, whereas among botanicals, Neem provided comparatively better performance to reduce the infestation. Moreover, a more comparative reduction in *T. absoluta* infestation on leaves was recorded during the 2nd spray of the various insecticide treatments than the 1st spray. The corrected infestation reduction of *T. absoluta* on leaves recorded with Flubendiamide after 24-hours of application was 36.07% that reached to 73.34% at the end of week, whereas the infestation reduction in Spinosad increased from 31.32% after 24-hours to 70.58% after one-week. Moreover, at the end of one week, the lowest infestation reduction was observed with Eucalyptus (53.65%), followed by Datura (54.54%), Tobacco (55.42%), Peppermint (56.64%), and Neem (58.41%). Among synthetic insecticides, Cyromazine was found to be the least effective with percentage reduction of *T. absoluta* infestation (66.59%) on tomato leaves, followed by Bifenthrin (67.70%) and Bifenthrin + Abamectin (68.81%).

3.3. Performance of Various Insecticides to Manage Infestation of *Tuta absoluta* on Tomato Fruits after First Spray

Figure 5 describes the results for the performance of the first spray of various synthetic and botanical insecticides on the mean infestation percentage of *T. absoluta* on tomato fruits. The pre-spray observations on *T. absoluta* infestation in various treatments exhibited a non-significant (F = 0.09, P = 0.9999) difference which ranged between 12.30 ± 1.39 to 13.40 ± 1.35% among the treatments. However, a significant (F = 4.24, P < 0.001)

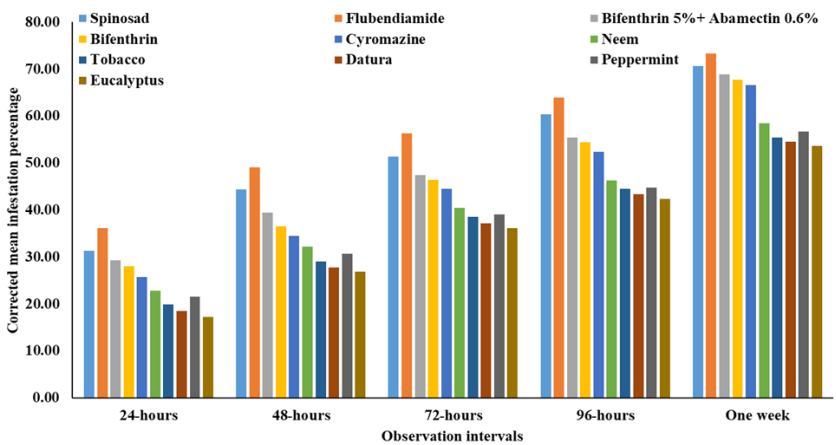

Fig. 4. Impact of various synthetic and botanical insecticides on the corrected mean infestation reduction percentage of *Tuta absoluta* on tomato leaves after the 2nd spray.

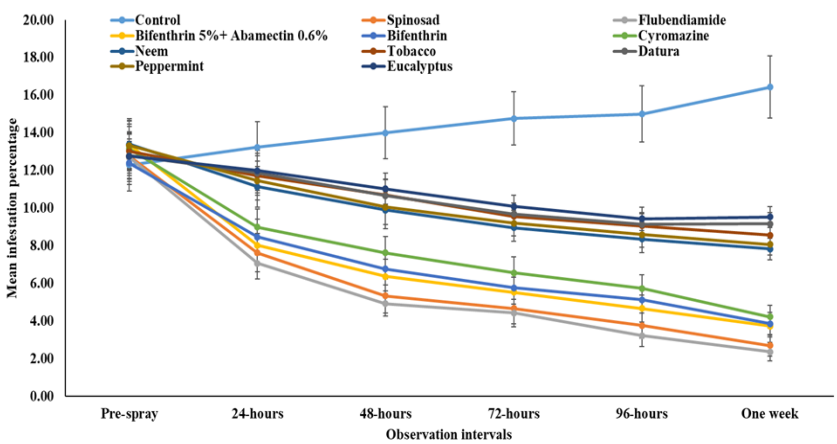

Fig. 5. Impact of various synthetic and botanical insecticides on the mean infestation percentage of *Tuta absoluta* on tomato fruits after 1st spray.

difference in the performance of various treatments was recorded after 24-hours of their application to reduce *T. absoluta* infestation on fruits. Flubendiamide (7.07 ± 0.84%) and Spinosad (7.63 ± 1.02%) were found to be the two most effective synthetic insecticides in infestation reduction of *T. absoluta* on fruits, followed by Bifenthrin + Abamectin (8.03 ± 1.00%), Bifenthrin (8.47 ± 0.94), and Cyromazine (9.00 ± 0.94%). Among botanical insecticides, Neem was found to be the most effective with the lowest infestation (11.13 ± 1.09%) of *T. absoluta* on tomato fruits, followed by Peppermint (11.47 ± 1.04%), whereas Eucalyptus (12.00 ± 1.07%) was recorded as the least effective among all the insecticides. The effectiveness of all the insecticides to reduce *T. absoluta* infestation on tomato fruits continued till the end of observations i.e., one week after their application but there was a significant difference among their performance at various observation intervals i.e., 48-hours ($F = 9.95$, $P < 0.001$), 72-hours ($F = 14.50$, $P < 0.001$), 96-hours ($F = 19.68$, $P < 0.001$), and one-week ($F = 32.23$, $P < 0.001$). At the end of week, the lowest *T. absoluta* infestation (2.37 ± 0.49%) was observed with Flubendiamide treatment, followed by Spinosad (2.70 ± 0.55%), whereas the infestation recorded in Bifenthrin + Abamectin (3.73 ± 0.57%), Bifenthrin (3.87 ± 0.59%), and Cyromazine (4.23 ± 0.61%) treatments was not-significantly different from each other. After one week, Neem was found to be the most effective botanical insecticide with mean *T. absoluta* infestation of 7.83 ± 0.56% on fruits, whereas Eucalyptus (9.53 ± 0.55%) and Datura (9.17 ± 0.59%) were found to be the least effective insecticides in infestation reduction on fruits. Overall, the highest *T. absoluta* infestation on tomato fruits was recorded in control at various observation intervals.

The corrected percentage reduction in *T. absoluta* on tomato fruits due to the first application of various synthetic and botanical insecticides is given in Figure 6. All the applied insecticides showed their effectiveness to reduce the *T. absoluta* infestation of tomato fruits but with significant differences as synthetic insecticides were found more effective than the botanicals. As per results, 24-hours after the application, Flubendiamide (46.60%) and Spinosad (42.32%) reduce the maximum infestation of *T. absoluta* followed by Bifenthrin + Abamectin (39.29%), Bifenthrin (36.02%), and Cyromazine (31.99%). The minimum infestation reduction percentage (9.32%) was recorded with Eucalyptus, followed by Datura (10.33%), whereas Neem (15.87%) was recorded as the most effective botanical to reduce *T. absoluta* infestation on fruits. Afterwards, a continuous reduction in *T. absoluta* infestation was observed in all the insecticide treatments at various time intervals i.e., 48-hours, 72-hours, 96-hours, and one week of their application. After one week, Flubendiamide (85.60%) and Spinosad (83.57%) emerged as the two most effective insecticides with respect to infestation reduction of *T. absoluta* on tomato fruits, whereas Eucalyptus (41.99%), Datura (44.22%), and Tobacco (47.87%) were found to be the least effective. Moreover, Bifenthrin + Abamectin (77.28%), Bifenthrin (76.47%), and Cyromazine (74.24%) was found to be equally effective in reduction of *T. absoluta* infestation on tomato fruits, whereas Neem (52.33%) was found to be most effective botanical, followed by Peppermint (50.91%).

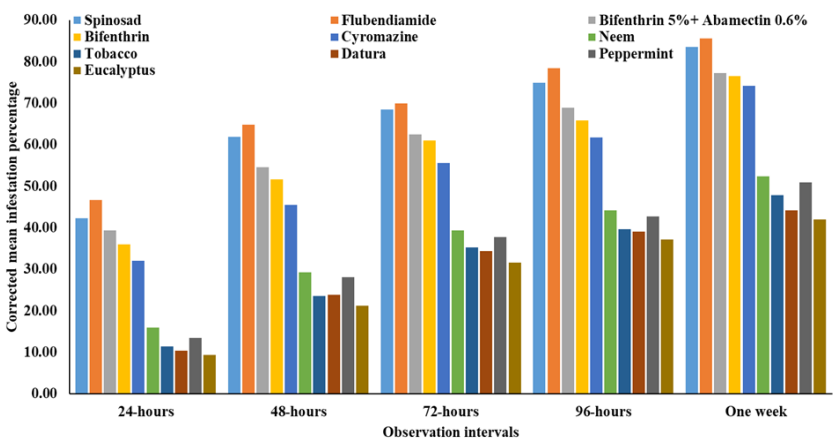

Fig. 6. Impact of various synthetic and botanical insecticides on the corrected mean infestation reduction percentage of *Tuta absoluta* on tomato fruits after 1st spray.

3.4. Performance of Various Insecticides to Manage Infestation of *Tuta absoluta* on Tomato Fruits after Second Spray

Figure 7 illustrates results for the effect of second spray of various insecticides on the infestation percentage of *T. absoluta* on tomato fruits. As compared to first spray, comparatively higher *T. absoluta* infestation (ranged between 13.40 ± 1.31% to 14.97 ± 1.49% among various treatments) was recorded on fruits with non-significant (F = 0.13, P = 0.9995) differences in the infestation among various insecticide treatments. However, 24-hours after application of various insecticides, there was a significant (F = 4.15, P < 0.001) difference in their effectiveness to reduce *T. absoluta* infestation on tomato fruits. Thus, Flubendiamide was found to be the most effective insecticide with the lowest *T. absoluta* infestation (7.13 ± 0.83%) on fruits, followed by Spinosad (7.97 ± 1.01%) and Bifenthrin + Abamectin (9.07 ± 0.92%), whereas Eucalyptus (12.03 ± 1.17%) and Datura (12.00 ± 1.03%) were found to be the least effective. Among botanical insecticides, Neem performed comparatively better with *T. absoluta* infestation on fruits of 11.57±1.09%, followed by Tobacco (11.83 ± 1.05%). The reduction in *T. absoluta* infestation on fruits due to the insecticide application was observed to continuously decline at various observation intervals up to one week. However, a significant difference in the performance of various insecticides was observed with respect to the level of infestation on fruits at various observation intervals i.e., 48-hours (F = 9.42, P < 0.001), 72-hours, 96-hours (F = 21.50, P < 0.001), and one week (F = 34.18, P < 0.001). Accordingly, after one week of the application of various synthetic and botanical insecticides, the lowest infestation percentage (2.00 ± 0.41%) of *T. absoluta* on tomato fruits was recorded with Flubendiamide, followed by Spinosad (3.00 ± 0.53%) and Bifenthrin + Abamectin (3.53 ± 0.57%), whereas Eucalyptus (10.17 ± 0.69%) and Datura (9.20 ± 0.68%) suffered the highest infestation. Among botanicals, Neem (7.47 ± 0.63%) was found to be the most effective, followed by Peppermint (8.30 ± 0.59%) and Tobacco (8.70 ± 0.54%). Overall, the highest *T. absoluta* infestation on tomato fruits was recorded in control at various observation intervals.

Figure 8 describes the results regarding the corrected infestation reduction of *T. absoluta* on tomato fruits after the second spray of various synthetic and botanical insecticides. The results indicated that all the synthetic insecticides were found significantly more effective to reduce *T. absoluta* infestation on fruits than the botanicals. A gradual increase in the infestation reduction of *T. absoluta* was observed since the application of insecticides that continued till the end of week. Accordingly, at the end of week, the highest corrected *T. absoluta* infestation reduction (88.00%) on fruits was recorded with Flubendiamide, whereas Spinosad and Bifenthrin + Abamectin caused 82.00 and 78.80% reduction in infestation, respectively. Among botanicals, Neem reduced 55.20% infestation of *T. absoluta* on tomato fruits, followed by Peppermint (50.20%). Overall, Eucalyptus and Datura were found to be the least effective among the evaluated insecticides against *T. absoluta* with corrected percentage reduction of 39.00 and 44.80%, respectively on tomato fruits.

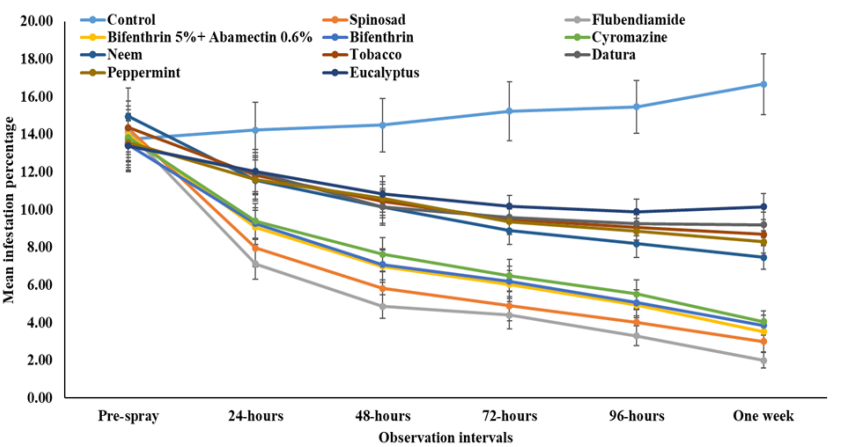

Fig. 7. Impact of various synthetic and botanical insecticides on the mean infestation percentage of *Tuta absoluta* on tomato fruits after the 2nd spray.

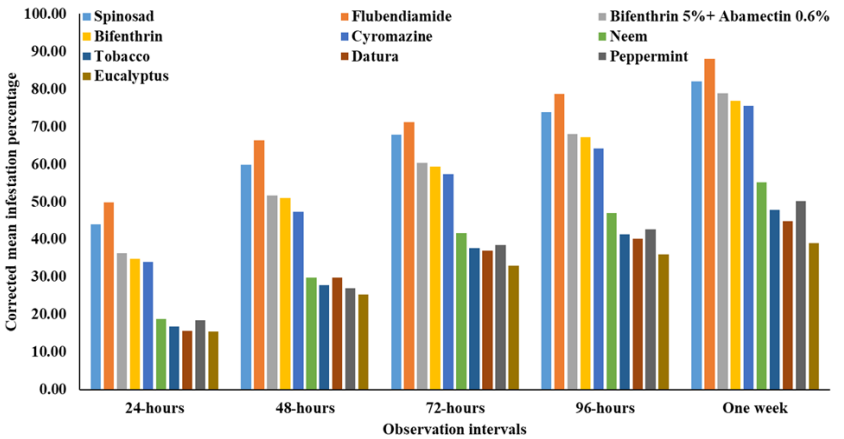

Fig. 8. Impact of synthetic and botanical insecticides on the corrected mean infestation reduction percentage of *Tuta absoluta* on tomato fruits after the 2nd spray.

3.5. Impact of Application of Various Insecticides on Tomato Yield in Response to Management of *Tuta absoluta* Infestation

The yield recorded in various insecticide treatments is given in Figure 9 which confirmed a highly significant ($F = 27.80$, $P < 0.001$) impact of *T. absoluta* infestation on tomato leaves and fruits on its yield. According to the results, the highest tomato yield was recorded in Flubendiamide (433.40 ± 5.46 maunds per acre) that was not significantly different from the yield obtained in Spinosad (420.80 ± 3.20 maunds per acre). Overall, the lowest yield (350.60 ± 5.89 maunds per acre) was recorded in control, followed by Eucalyptus (366.80 ± 4.21 maunds per acre). Moreover, tomato yields recorded in Bifenthrin + Abamectin (408.80 ± 5.33 maunds per acre), Bifenthrin (400.40 ± 3.83 maunds per acre), and Cyromazine (397.60 ± 4.71 maunds per acre) was not significantly different from each other. Among botanical insecticide treatments, the highest yield (389.60 ± 4.86 maunds per acre) was recorded in Neem, whereas yield recorded in Peppermint (380.20 ± 3.32 maunds per acre), Tobacco (375.20 ± 4.40 maunds per acre), and Datura (371.80 ± 5.37 maunds per acre) were not significantly different from each other.

4. DISCUSSION

The devastation caused by *T. absoluta* in various tomato-growing areas of the world necessitates effective measures to reduce its losses [36]. Accordingly, pesticides, both conventional and botanical, are widely used to keep *T. absoluta* populations below threshold levels, however repeated application of these pesticides is required [2, 37]. However, due to the ever increasing and injudicious use of synthetical chemicals against *T. absoluta* has resulted in the death of its natural

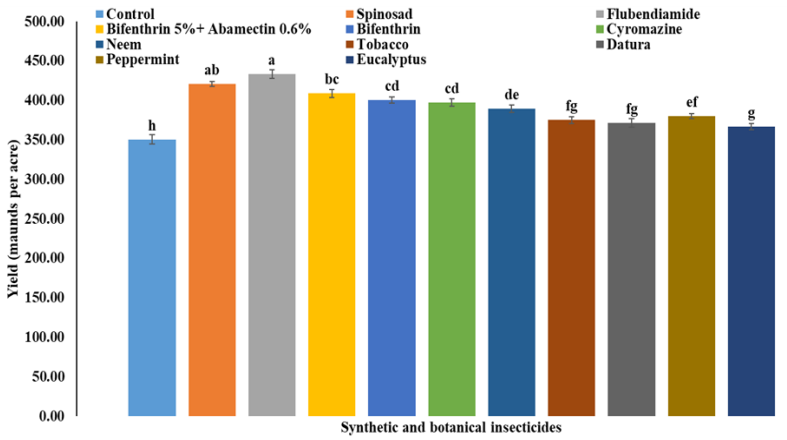

Fig. 9. Impact of synthetic and botanical insecticides used against *Tuta absoluta* on tomato fruit yield.
*Means followed with same letters are not significantly different (LSD = 13.331, $P < 0.05$).

enemies, development of resistant, environmental pollution, human health hazards, and residues in tomatoes which are mostly consumed fresh [38, 39]. Accordingly, there is an increasing trend in the use of biorational and botanical insecticides for the management of *T. absoluta* in field and greenhouse tomato orchards [40]. In continuity of the same, different synthetic and botanical insecticides evaluated in this study against *T. absoluta* were found effective in reducing its infestation on tomato leaves and fruits but differ significantly with respect to the level of control they provided against the pest. Synthetic insecticides i.e., Flubendiamide, Spinosad, Bifenthrin + Abamectin, Bifenthrin, and Cyromazine showed significantly better performance than botanical pesticides i.e., Neem, Tobacco, Datura, Peppermint, and Eucalyptus to reduce the infestation of *T. absoluta.* Both Flubendiamide and Spinosad were able to reduce *T. absoluta* infestation on leaves and fruits up to 73.34 and 88.00%, and 70.58 and 83.57%, respectively, whereas the remaining three synthetic insecticides were also capable to reduce more than 50% infestation of *T. absoluta* on tomato leaves and fruits. Overall, botanical pesticides were not so effective to reduce the infestation of *T. absoluta* in tomato, however, Neem and Peppermint were found to be comparatively more effective.

Considering synthetic insecticide as the most reliable method of managing *T. absoluta*, a lot of research has been done to evaluate various groups of insecticides to reduce their losses in tomato. In a field study, while evaluating eleven synthetic insecticides, significant performance of Spinetoram, Cyantraniliprole, Flubendiamide and Spinosad was observed to control *T. absoluta* infestation on tomato leaves and fruits [41]. In another study, comparatively higher toxicity of Spinosad against third instar *T. absoluta* larvae was recorded followed by Emamectin benzoate + Lufenuron and Flubendiamide, however, it was also highly toxic to *Trichogramma* sp. than Flubendiamide [42]. Another study of Kumar *et al.* [43] also recorded better performance of new chemistry insecticides i.e., Chlorantraniliprole, Spinosad, and Flubendiamide to control variable population of *T. absoluta* in Tamil Nadu, India. Moeini-Naghade *et al.* [44] while evaluating the efficacy of five different groups of pesticides i.e., Spinosad, Abamectin, Imidacloprid, Indoxacarb, and Cypermethrin against immature (eggs, larvae) and adults of *T. absoluta* found that none of the insecticides were found effective against the adult *T. absoluta* except Spinosad with 40% efficacy. However, Abamectin and Imidacloprid were the most and least effective insecticides against the targeted eggs. Moreover, Abamectin with 0.45 mg active ingredient (ai) per liter of LC_{50} was found to be the most effective against 3rd instar larvae, whereas Imidacloprid was found to be the least effective against the larvae. Hence, both Spinosad and Abamectin were recommended for their use to manage *T. absoluta* losses in the field.

Kandil *et al.* [36] studies under laboratory conditions found comparable performance of Spinosad along with Bt having Lepinox with synthetic insecticides (Chlorpyrifos and Indoxacarb) in causing significant mortalities among targeted 2nd instar *T. absoluta* larvae. The same studies also reported that the application of Bt formulation and Emamectin benzoate can increase the larval duration and cause pupal mortality, hence resulting in lower adult emergence, beside various abnormalities recorded in individuals of various life stages. The significant performance of Emamectin benzoate and Chlorantraniliprole to cause significant *T. absoluta* mortality was recorded under laboratory conditions, followed by Spinetoram and Abamectin Kandil et al. [45]. Moreover, under field trials, both Emamectin benzoate and Chlorantraniliprole also reduce 95.51 and 98.74% infestation of *T. absoluta* on tomatoes [46].

The findings of the above-mentioned studies mostly supported our findings as all the evaluated insecticides were effective in reducing the infestation of *T. absoluta*, especially Belt (Flubendiamide), Tracer (Spinosad) along with Novastar (Bifenthrin 5%+ Abamectin 0.6%). The higher efficiency of these insecticides against *T. absoluta* may be due to their novel mode of action (ryanodine receptor agonist, neuronal hyperexcitation and neurotoxic) and no or lower level of resistance among the targeted *T. absoluta* populations, as it has recently been introduced in Pakistan [31-33]. It has been mentioned that during 1990's, organophosphates and pyrethroids were frequently used for the management of *T. absoluta* in various countries of the world, but soon the pest developed resistance against most of these pesticides [15, 34], and the same led to the development and use of new chemical insecticides

against it. Therefore, the results obtained in our study are supported by the findings of many studies where new chemical insecticides exhibited a significant impact on the infestation reduction of *T. absoluta*. In addition to the above studies, the higher efficacy of Flubendiamide, Emamectin benzoate, Chlorantraniliprole, and Spinosad against *T. absoluta* was observed by Roditakis *et al.* [46] as their LC_{50} values ranged between 0.03 to 0.53 ppm, whereas Chlorpyriphos and Cypermethrin were found to be the least effective with LC_{50} values between 475 to 2038 ppm. Similarly, Roby and Hussein [47] also recorded higher efficacy of Emamectin benzoate against *T. absoluta,* whereas more than 90% mortality of the pest was observed using Spinosad and Emamectin benzoate [48]. Moreover, while evaluating 11 insecticides against *T. absoluta*, Sridhar *et al.* [41] found Spinetoram, Cyantraniliprole, and Spinosad as the most effective insecticides.

In our study, only two sprays were required to reduce *T. absoluta* infestation to the acceptable threshold levels and the same may be attributed towards the new introduction or less intensity of the pest in the study area. However, generally frequent applications of insecticides are required to reduce the damage of *T. absoluta* [37] as studies suggested that up to 36 sprays in Brazil [49] were conducted to keeps its infestation below threshold levels, whereas 15 applications are reported from Spain [50]. Moreover, an alarming rise in the use of insecticides has been witnessed in Europe since the arrival of *T. absoluta* in the continent [51] which ultimately poses great threats to humans, the environment, and the non-target organisms [19].

Despite huge amounts of insecticides used against *T. absoluta,* mostly they failed to get desired results because of the endophytic nature of larvae to feed (feeding in the mesophyll of leaves), that make it difficult for chemicals to reach and kill the targeted larvae [52]. Moreover, it has also the ability to quickly develop resistance against most commonly used insecticides [14, 50] such as Cartap, Abamectin, and pyrethroids [12, 13], organophosphates, Spinosad, Emamectin Benzoate, Indoxacarb, Flubendiamide, Spinetoram, Cyantraniliprole, and Abamectin [36, 53], chloride channel activators, benzoylureas [54], and diamides [16, 17]. Accordingly, the insecticide resistant populations of *T. absoluta* have already been reported from Italy, Greece, and Israel [50]. Therefore, it is always suggested as a prudent practice that a rotation in the use of active ingredients should be practiced against any insect pests, including *T. absoluta* to reduce the chances of resistance development in the pest. In this way, the suggested rotation of active ingredients against *T. absoluta* for its better management include Imidacloprid, Indoxacarb, Spinosad, Deltamethrine (against adult moths) and Rynaxypyr [2]. In addition to rotation in the use of different insecticide groups against *T. absoluta*, the use of various botanical insecticides has also shown an increase during the 21st century keeping in view the health and environmental hazards of the synthetic insecticides [55].

Accordingly, in addition to synthetic insecticides, we also evaluated aqueous extracts of Neem, Tobacco, Peppermint, Datura, and Eucalyptus to determine their effectiveness in infestation reduction of *T. absoluta.* Although botanicals were not so effective as that of synthetic insecticides, Neem, Peppermint, and Tobacco aqueous extracts exhibited some promising results to reduce *T. absoluta* infestation on tomato leaves and fruits. Among botanicals, Neem and Peppermint were able to reduce up to 58.41 and 55.42% *T. absoluta* infestation on tomato leaves, whereas reduction in infestation on fruits was observed as 55.20 and 50.20%, respectively. The lower efficiency of botanicals observed in the study may be due to the fact that most of them have contact mode action resulting in antifeedant or repellency of the pests [56], whereas *T. absoluta* larvae possess cryptic nature of damage as it mostly remained concealed within tomato leaves and fruits, hence require insecticides with systematic mode of action to get its better management [57]. Despite lower performance of botanicals recorded in our study, promising results of various botanical and biorational insecticides, either commercial formulations or extracted in organic solvents, have been observed on mortality and infestation reduction of *T. absoluta* in tomatoes [40]. Among botanicals, Neem extracts or their commercial formulations have shown promising results on mortality and reduction of *T. absoluta* infestation in tomatoes [23, 58]. Kona *et al.* [23] recorded 24.5% and 86.70 to 100% mortality of the targeted *T. absoluta* eggs and larvae, respectively with the application of Neem extracts, whereas 25% and 87 to 100% mortality was recorded with Jatropha extracts within four

days of the exposure. However, both extracts were unable to affect the viability of the treated eggs as all the remaining eggs hatched after four-days of exposure. Among other plants i.e., Basil, Garlic, Thyme, Castor Bean, Eucalyptus, Chinaberry, Onion, and Geranium also exhibited insecticidal potential against *T. absoluta* larvae, however their effectiveness varied significantly [59, 60].

Among other studies which identify the insecticidal potential of Neem and other botanical or biorational pesticides against *T. absoluta,* Buragohain *et al.* [40] reported that various Neem (Ecotin and EcoNeem) and *Bacillus thuringiensis* (Green Larvicide and Delfin) were found quite effective against the pest as their performance was comparable with that of commercial insecticide (Chlorantraniliprole). The findings of Jallow *et al.* [61] also supported our study results as 70-86% mortality of 2nd instar *T. absoluta* larvae was recorded under laboratory conditions when they were provided with leaves dipped with commercial Azadirachtin formulation at 3 grams per liter. Moreover, they also observed significant reduction in the *T. absoluta* infestation under field conditions with the use of Neem along with *Bt* and *Beauveria bassiana* commercial formulations. Similarly, Pires *et al.* [62] and Mollá *et al.* [63] also recorded significant impact of Neem and biorational insecticide on infestation reduction of *T. absoluta* on tomato. Comparatively higher efficiency of Neem extracts was recorded against *T. absoluta* under both laboratory and field conditions, followed by Garlic-Clove, Lemon, Bishkathali and Mahogany extracts, resulting in higher yields as compared to the control [64].

Although Neem or other botanicals have shown insecticidal potential against *T. absoluta*, there is a great variation in their effectiveness, particularly the home or field made formulations because of their inferior quality and standardization [2]. Moreover, Azadirachtin of the Neem extracts readily breaks down or isomerizes under sunlight due to its high photosensitivity, thus has low residue under field conditions and requires frequent application to manage insect pests including *T. absoluta* [58]. Another reason for the less adoptability of Neem-based commercial formulation is their cost that is estimated at US$ 12 to 15 per liter along with repeated application, hence making it difficult for small farmers to use [2].

In the study undertaken, the performance of botanical pesticides was significantly lower than the synthetics, and the same is in accordance with the findings of Hosseinzadeh and Aramideh [65], who reported that Thiocyclam and Spinosad were found to be more effective than Neem and Bt based insecticides against *T. absoluta* larvae. However, in a recent study, Taleh *et al.* [66] evaluated four commonly used insecticides i.e., Emamectin Benzoate, Imidacloprid, Lambda-Cyhalothrin, and Thiacloprid, along with biopesticide (Azadirachtin) against 2nd instar *T. absoluta* larvae using leaf-dipping methods to determine their effect on the survival and various growth parameters. The results indicated that Emamectin benzoate and Azadirachtin were found to be most effective in causing significant mortality and adversely affecting various growth parameters. Thus, pre-oviposition adult period, adult longevity and fecundity were significantly less in Emamectin benzoate treatment, whereas Emamectin benzoate and Azadirachtin also reduced the survivability of the 4th instar larvae.

5. CONCLUSIONS

All the synthetic insecticides were found to be more effective in reducing the infestation percentage of *T. absoluta* on tomato leaves and fruits, however there was a significant difference among them. Flubendiamide and Spinosad were the two best insecticides, whereas Bifenthrin + Abamectin, Bifenthrin, and Cyromazine were also able to reduce considerable reduction in *T. absoluta* infestation. Among botanicals, Neem and Peppermint were the two most effective botanicals. Therefore, considering infestation of *T. absoluta*, spray of Neem extracts should be done on a regular basis to keep its infestation under control. However, if required, application of synthetic insecticides, i.e., Flubendiamide or Spinosad should be used as the last option.

6. CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Research Article

Phytochemical and Antibacterial Activity of *Aloe vera, Camellia sinensis* and *Momordica charantia*

Tahreem Shahzadi, Sana Zahoor*, Arooba, and Omama Afzal

Department of Biological Sciences, Virtual University of Pakistan, Lahore, Pakistan

Abstract: Traditional medicines, largely derived from plants, contain bioactive compounds that serve as protective agents against environmental stressors (biotic and abiotic) and can also enhance human health. With the alarming rise in antimicrobial resistance, there is an urgent need to explore safe and effective plant-derived alternatives to synthetic antibiotics. The present study was therefore designed to evaluate the phytochemical composition and antibacterial potential of three commonly used medicinal plants: *Camellia sinensis*, *Aloe vera*, and *Momordica charantia*. Aqueous and ethanolic extracts were prepared and qualitatively screened for tannins, alkaloids, saponins, flavonoids, steroids, proteins, carbohydrates, phlobatannins, and terpenoids. Quantitative analysis further revealed that *M. charantia* had the highest alkaloid content (5.29%), while its ethanolic extract exhibited the greatest protein (37.9 mg/g dry extract) and carbohydrate levels (30.6 mg/g dry extract). The antibacterial activity of the extracts was tested against a Gram-positive strain (*Staphylococcus aureus*) and a Gram-negative strain (*Klebsiella pneumoniae*). The results showed that *C. sinensis* consistently demonstrated the strongest antibacterial activity in both aqueous and ethanolic extracts, producing inhibition zones of 20 ± 0.95 mm against *S. aureus* and 12 ± 2 mm against *K. pneumoniae*. In comparison, *M. charantia* exhibited selective inhibition, being effective only against *S. aureus*, while *A. vera* showed moderate antibacterial activity depending on the extraction solvent. Overall, the findings highlight the therapeutic promise of *C. sinensis* as a natural antimicrobial agent. Future studies should expand antimicrobial screening to additional pathogens and include in vivo assays to validate the clinical applicability of these extracts.

Keywords: Phytochemicals, Qualitative Analysis, Quantitative Analysis, Antibacterial Activity, Zone of Inhibition, Phytotherapy.

1. INTRODUCTION

Nature has long been recognized for its positive effects on human health, largely due to the presence of plants and their bioactive compounds. Plants produce diverse secondary metabolites, collectively known as phytochemicals, which are not essential nutrients but play critical roles in disease prevention and therapeutic applications [1-3]. These compounds help modulate oxidative stress, regulate immune responses, and exert antimicrobial effects, making them promising alternatives to synthetic antibiotics in the time of rising drug resistance [4-6].

Phytochemicals are grouped into several classes, each with distinct biological activities. Flavonoids, found in vegetables, fruits, tea, and red wine, are well-documented for their antioxidant, anti-inflammatory, and anticancer effects [7]. Alkaloids, nitrogen-containing compounds such as caffeine in coffee or theobromine in cocoa, display analgesic, antimicrobial, and anti-inflammatory properties [8]. Saponins, abundant in legumes and beans, possess immunomodulatory and anticancer activity [9, 10]. Terpenoids from aromatic plants like mint and citrus fruits display antimicrobial and antioxidant effects [11]. Phytosterols in seeds and nuts help regulate cholesterol metabolism and induce apoptosis in tumor cells [12].

In South Asian traditional medicine, plants such as Bitter gourd (*Momordica charantia*), *Aloe vera*, and Green tea (*Camellia sinensis*) are frequently used to manage infections, metabolic disorders, and inflammatory conditions.

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* Corresponding Author: Sana Zahoor <sana.zahoor@vu.edu.pk>

However, despite their well-documented ethnopharmacological value, systematic comparisons of their phytochemical content and antibacterial efficacy against clinically important pathogens remain limited [13].

Bitter gourd is widely known for its hypoglycemic effects, mediated by compounds such as charantin, momordicins, and vicine [14-16]. Beyond its metabolic role, recent studies have reported that extracts of *M. charantia* exhibit strong antibacterial activity against a range of pathogens, including *Staphylococcus aureus* and periodontal bacteria, while also displaying antioxidant and anti-inflammatory properties [17-19].

Aloe vera contains polysaccharides, anthraquinones, glycoproteins, and antioxidants that contribute to its anti-inflammatory, wound-healing, and skin-protective activities [20-22]. Importantly, *A. vera* extracts have also demonstrated antimicrobial activity against a variety of clinical isolates, suggesting its potential as a complementary therapeutic agent [22].

Green tea (*Camellia sinensis*) is particularly rich in catechins. These compounds are potent antioxidants and have been associated with cardiovascular and metabolic health benefits [23-25]. In addition, in vitro studies have revealed that green tea extracts exhibit antibacterial activity against pathogens including *S. aureus* and MRSA [26, 27], highlighting its potential dual role in both preventive and therapeutic health strategies.

Despite such promising findings, there is still limited research that directly compares these plants' phytochemical composition with their antibacterial efficacy against clinically relevant pathogens. Therefore, the present study was designed to evaluate and compare the antibacterial activity of bitter gourd, *Aloe vera*, and green tea extracts against *Staphylococcus aureus* and *Klebsiella pneumoniae*, and to correlate these activities with the presence of key phytochemicals (alkaloids, saponins, etc.). By employing both aqueous and ethanol extracts, this work highlights the novelty of a comparative phytochemical–antibacterial approach in the Pakistani context, addressing an urgent need for safer plant-based alternatives to conventional antibiotics.

2. MATERIALS AND METHODS

2.1. Sample Preparation

Sample plants (*Momordica charantia* (leaves), *Aloe vera* (skin) and *Camellia sinensis* (leaves and buds) were collected from the local markets in Gujranwala, Pakistan and then washed with tap water, dried under open air and sunlight till completely dry and ground into fine powder by using electric grinder.

2.1.1. Extraction

Solvents (ethanol and distilled water) were used for extraction. Powdered samples (3 g) were soaked in each of the solvent separately. The samples were kept for 4 hours in the water bath to ensure complete dissolution of chemicals and then kept on orbital shaker for 12 hours; filtered and stored at 4ºC for further analysis [23].

2.2. Qualitative Phytochemical Analysis

2.2.1. Test for tannins

4-6 drops of 5% ferric chloride were added to 2 ml of the sample extract. The hydrolyzed tannins can be detected by observing the color changes to blue-black [28].

2.2.2. Test for flavonoids

2% NaOH solution was added to test tubes containing the sample (1 ml). 3-4 drops of dilute HCl were added. Deep yellow color that disappears within seconds indicates a positive test [29].

2.2.3. Test for alkaloids

Sample extract (1 ml) was taken in tube and mixed with 2 ml of conc. HCl and boiled at 100 °C in a water bath for 5 minutes. 4-6 drops of Wagner's reagent were added to the sample. Reddish-brown precipitate indicates positive result [28].

2.2.4. Test for saponins

Sample extract (2 ml) was tested for foam formation by shaking the tube vigorously for almost 30 seconds. Foam formation confirms the presence of Saponins [30].

2.2.5. Test for steroids

Chloroform (2 ml) was added to a 1 ml sample extract. A few (3-4) drops of concentrated H_2SO_4 were added alongside the walls of the test tube. The red ring at inter-phase indicates positive test [29].

2.2.6. Test for protein

Sample extract (2 ml) was boiled with few drops of ninhydrin solution (1%). Violet-blue colour (known as Ruhemann's purple) confirms the presence of protein [31].

2.2.7. Qualitative test for carbohydrates

a) Molisch test: Ten drops of Molisch reagent were added to 2 ml of the extract. Later, concentrated H_2SO_4 was added along the sides of the test tube. The violet ring at the inter-sectional plane indicates positive test [28].
b) Benedict test: Crude plant extract (1 ml) was added to Benedict's reagent (1 ml) and boiled. Red precipitates demonstrate the occurrence of reducing carbohydrates [28].

2.2.8. Test for phylobatannins

Liquid gelatin was added to sample extract (2 ml). Creamy texture indicates the presence of phylobatannins [28].

2.2.9. Test for terpenoids

Crude extract (1 ml) was added to 2.0 ml of chloroform in a test tube. Concentrated H_2SO_4 (2 ml) was added to the solution and the solution was boiled for 2 minutes. Reddish brown color indicates presence of terpenoids [32].

2.3. Quantitative Phytochemical Analysis

2.3.1. Extract preparation

Plant samples (4 grams) were extracted individually with ethanol and water (40 ml). The extraction was carried out in a water bath at 25 °C for one hour. The samples were filtered, and the resulting extract was dried at 60 °C in a hot air oven. Dried extracts were then stored in amber-coloured glass bottles at 4 °C to prevent oxidative damage. Percent yield of the extracts was calculated using the following formula:

$$\text{Percent Yield (\%)} = \frac{W1}{W2} \times 100$$

Where,
W1 = Weight of the extract residue obtained after solvent removal
W2 = Weight of the plant powder used

2.3.2. Total alkaloids determination

The total alkaloid content was determined by adding 20 ml of 10% acetic acid in ethanol to 1 gram of the sample. The resulting solution was covered with foil and allowed to settle for four hours. The solution was filtered after 4 hours and the filtrate was boiled down in a water bath to reach 1/4 of the original volume. Concentrated ammonium hydroxide solution was then carefully added until the precipitation process was completed. The precipitates were then collected, rinsed with dilute NH_4OH (ammonium hydroxide), and then filtered. The residue was dried up to constant weight to determine total alkaloid content. In this study, alkaloids were determined from the plants leaves/ buds samples; total alkaloids were determined using the following formula:

$$\text{Alkaloids (\%)} = \frac{\text{mass of residue}}{\text{mass of sample}} \times 100$$

2.3.3. Total protein determination

Bradford reagent (2.5 ml) was added to a test tube containing 0.1 ml of extract. The test tube was allowed to stand for 5 minutes. Absorbance was noted at 595 nm. Bovine serum albumin (BSA) was used as standard and blank tube contained distilled water in place of sample [33].

2.3.4. Total carbohydrate determination

Sample extract (100 µl) was added to 1900 µl distilled water to make the total volume 2 ml. DNS (2 ml) was added, and the tubes were placed in boiling water for 5 minutes. Then 6 ml distilled water was added, and absorbance was recorded at 550 nm. In blank tube, sample extract was replaced with distilled water [34].

2.4. Antibacterial Activity

Antibacterial activity of *Momordica charantia, Camellia sinensis* and *Aloe vera* was observed against a Gram-positive (*Staphylococcus aureus*

ATCC 25923) and Gram-negative bacterium (*Klebsiella pneumoniae ATCC 13883*).

2.4.1. Inoculum preparation

Sterilized nutrient broth medium was used for inoculum development. A single bacterial colony of *Klebsiella pneumoniae* and *Staphylococcus aureus,* was separately inoculated into sterilized nutrient broth. Flasks were kept in the shaking incubator for ~24 hours. Flasks were kept in the shaking incubator at 37 °C, 150 rpm for 24 hours until OD600 = 0.5, corresponding to 1×10^8 CFU/ml. This inoculum was diluted to 1×10^6 CFU/ml before testing.

2.4.2. Determination of antibacterial activity

To check the antibacterial activity, wells were created in the petri plates with the help of sterilized tips. These wells were then loaded with the sample extracts (150 µl). The final concentration of the extract was standardized at 10 mg/150 µl (66.6 mg/ml), for antibacterial testing, based on preliminary assays and literature reports [35]. All experiments were performed in triplicate (n = 3) for each extract and control. Streptomycin (10 µg/ml) was used as a positive control, while distilled water and ethanol were used as negative controls. The inclusion of solvent controls was to account for any inhibitory effect of extraction solvents themselves. The plates were kept in the incubator at 37 °C for 24 hours. Zones of inhibition were measured in millimeters using a vernier caliper [36].

2.4.3. Statistical analysis

All data were analyzed using Costat statistical software (Snedecor and Cochran, 1980). Descriptive statistics (mean ± standard deviation) were calculated, One-way ANOVA was performed to assess significant differences among treatments, and means were separated using Tukey's HSD post-hoc test at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Physical Properties of Plant Extracts

The aqueous and ethanolic extracts of *Camellia sinensis*, *Aloe vera*, and *Momordica charantia* exhibited distinct colors and viscosities, reflecting differences in solvent polarity and phytochemical solubility (Table 1). Aqueous extracts generally appeared yellow to orange, while ethanolic extracts were green to olive in color, consistent with earlier reports that extraction solvents influence pigment and metabolite profiles [17, 37]. Ethanol and water were selected as extraction solvents due to their safety, availability, and complementary polarity ranges. Ethanol favors the extraction of relatively non-polar compounds such as alkaloids and flavonoids, while water is more efficient for polar constituents like tannins, carbohydrates, and proteins. This dual approach ensured a broad coverage of phytochemicals and reflects commonly used practices in pharmacognosy studies [38, 39].

3.2. Qualitative and Quantitative Phytochemical Analysis

Momordica charantia showed positive results for proteins, tannins, phylobatannins, steroids and alkaloids with both the extracts. Saponins and flavonoids were present in aqueous extract only and were absent in ethanol extract. Benedict's test for carbohydrates was negative for both extracts. Jia *et al.* [15] carried out the phytochemical screening of *Momordica charantia* and reported the presence of triterpenes, polysaccharides, saponins, proteins and flavonoids.

Table 1. Physical properties of different plant extracts.

Sample botanical name	Sample common name	Physical properties	Aqueous extract	Ethanol extract
Camellia sinensis	Green tea	Colour	Yellow brown	Dark green
		Viscosity	Viscous	Non-Viscous
Aloe vera	Aloe vera	Colour	Yellow	Dark green
		Viscosity	Viscous	Non-Viscous
Momordica charantia	Bitter gourd	Colour	Orange	Olive green
		Viscosity	Non-Viscous	Non-Viscous

Camellia sinensis showed positive results for carbohydrates, tannins, phylobatannins and alkaloids with both extracts and showed positive results for proteins, steroids and terpenoids with ethanol extract only and gave positive results for saponins with aqueous extract only. Both extracts gave negative results for flavonoids. The aqueous extract of *Aloe vera* gave negative results for proteins, carbohydrates, steroids, and terpenoids, while it gave positive results for tannins, flavonoids, phylobatannins, alkaloids, and saponins (Table 2). The variability of phytochemical content depends on the extraction method [40].

Qualitative screening confirmed the presence or absence of key phytochemicals, whereas quantitative assays provided numerical validation, thereby strengthening the reliability of the findings. Qualitative and quantitative analyses revealed variation in secondary metabolite content across species and solvents. Ethanol extract of *M. charantia* showed the least percentage yield of 4% while aqueous extract of *Aloe vera* showed the highest percentage yield (32.5%) as shown in Figure 1. *M. charantia* was particularly rich in alkaloids (5.29%), whereas *Aloe vera* gave the lowest (4.38%) alkaloid content (Figure 2). The ethanol extract of *M. charantia* gave the maximum carbohydrate concentration (30.6 mg/g dry extract), while the aqueous extract of *A. vera* contained the minimum protein (2.14 mg/g dry extract) (Figure 3). Similarly, protein analysis showed that the ethanol extract of *M. charantia* had the highest protein content (37.9 mg/g dry extract), in contrast to the aqueous extract of *A. vera*, which recorded the lowest protein content (1.45 mg/g dry extract) (Figure 4). These findings are comparable with earlier reports highlighting *M. charantia* as a source of bioactive triterpenoids and alkaloids [15]. The differences observed between aqueous and ethanol extracts align with solvent polarity: ethanol more efficiently extracts phenolics, terpenoids, and alkaloids, while water favors hydrophilic compounds such as polysaccharides [41].

3.3. Antibacterial Activity

Ethanol extract of *C. sinensis* produced the strongest inhibition against *S. aureus* (20 ± 0.95 mm), which can be attributed to its higher tannin and alkaloid contents (Table 3). Tannins from green tea are well-documented for disrupting bacterial membranes and enzyme activity [42]. In contrast, *A. vera* showed weaker activity, consistent with its lower alkaloid and protein levels. In the present study, negative controls (sterile water and ethanol) did not produce any inhibition zones, confirming that the observed antibacterial effects were solely due to the plant extracts.

Table 2. Qualitative phytochemical analysis.

Tests		***Momordica charantia***		***Camellia sinensis***		***Aloe vera***	
		Aqueous extract	**Ethanol extract**	**Aqueous extract**	**Ethanol extract**	**Aqueous extract**	**Ethanol extract**
Proteins		+++	++	-	++	-	+
Carbohydrates	Molisch Test	+	+++	+	+++	-	+
	Benedict's Test	-	-	+	+	-	+
Tannins		+	+++	+	+	+	+++
Flavonoids		+	-	-	-	++	-
Phylobatannins		+++	+	++	+	+	+
Steroids		+	+++	-	+++	-	++
Terpenoids		-	+++	-	+++	-	+
Alkaloids		+++	++	+++	+++	++	+++
Saponins		+++	-	+	-	+	-

Absent (-), Slightly Present (+), Moderately Present (++), High concentration (+++)

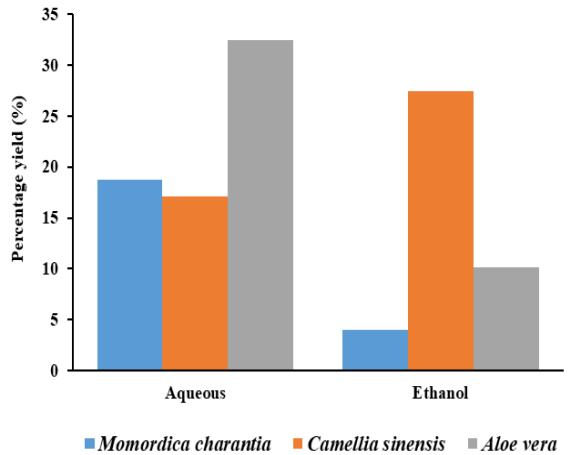

Fig. 1. Percentage yield of selected plants in aqueous and ethanol extracts.

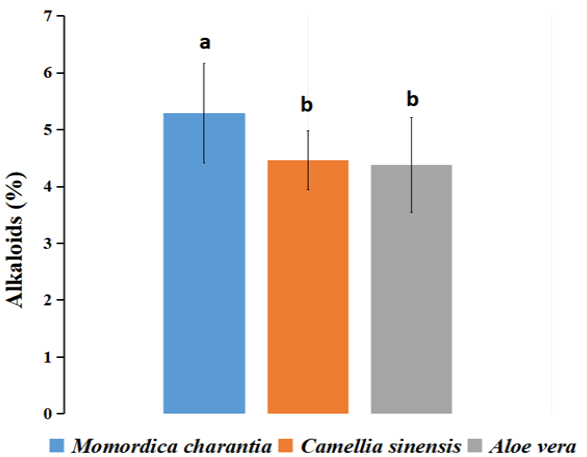

Fig. 2. Total alkaloid content in selected plants.
Error bars represent the standard deviation (± SD) among triplicate. Values with different superscript letters differ significantly ($p \leq 0.05$).

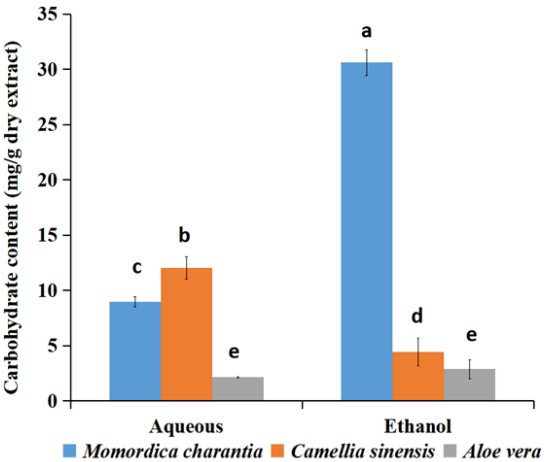

Fig. 3. Total carbohydrate content of selected plants in aqueous and ethanol extracts.
Error bars represent the standard deviation (± SD) among triplicate. Values with different superscript letters differ significantly ($p \leq 0.05$).

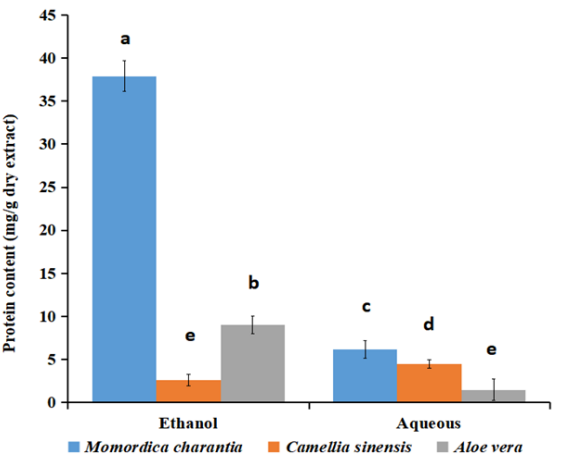

Fig. 4. Total protein content of selected plants in aqueous and ethanol extracts.
Error bars represent the standard deviation (± SD) among triplicate. Values with different superscript letters differ significantly ($p \leq 0.05$).

Table 3. Antibacterial activity.

Sample	Extract	Zone of Inhibition (mm)	
		Staphylococcus aureus	*Klebsiella pneumoniae*
Momordica charantia	Ethanol	15 ± 0.45^{c}	0.0 ± 0^{d}
	Aqueous	0.0 ± 0^{d}	0.0 ± 0^{d}
Camellia Sinensis	Ethanol	20 ± 0.95^{b}	12 ± 0.2^{b}
	Aqueous	10 ± 0.57^{c}	11 ± 0.14^{c}
Aloe vera	Ethanol	11 ± 0.1^{c}	12 ± 0.28^{b}
	Aqueous	0.0 ± 0^{d}	0.0 ± 0^{d}
Streptomycin		29 ± 0.36^{a}	24 ± 1.2^{a}

Anita *et al.* [26] reported a zone of inhibition (12.66 mm) for the ethanolic extract of *C. sinensis* against *L. acidophilus*. Sowjanya *et al.* [27] studied the antimicrobial activity of *C. sinensis* against *K. pneumoniae* and *S. aureus*, and determined the minimum bactericidal concentration (MBC) as 5 mg/ml for both strains. Ethanol extracts of *C. sinensis* and *Aloe vera* gave a zone of inhibition (12 ± 0.2 mm) against *K. pneumoniae* while aqueous extract of *C. sinensis* gave a zone of inhibition (11 ± 0.14 mm). Ethanolic extract of *M. charantia* exhibited low activity against *K. pneumoniae* (MIC = 625 µg/ml) [18]. Khalid *et al.* [19] tested the antibacterial activity of leaves of *M. charantia,* and the results showed that 80% methanolic extract exhibited the highest antibacterial potential against with inhibition zone diameters of 30 mm against *P. multocida* and 28 mm against *A. parasiticus,* respectively. Aqueous control showed no zone of inhibition, whereas ethanolic control showed a very minimum zone (3-4 mm).

4. CONCLUSIONS

This study demonstrated that the phytochemical composition of *Momordica charantia, Aloe vera,* and *Camellia sinensis* influenced their antibacterial activity, with *C. sinensis* showing the strongest and most consistent effects. *M. charantia* was effective mainly against *S. aureus* and *A. vera* showed moderate activity; whereas *C. sinensis* exhibited broad antibacterial potential, highlighting its promise as a natural therapeutic agent. However, the study was limited to two bacterial strains; future work should include additional Gram-positive, Gram-negative, and fungal pathogens, along with MIC/MBC, antioxidant, and phenolic assays to better elucidate antimicrobial mechanisms and therapeutic relevance.

5. CONFLICT OF INTEREST

Authors declare no conflict of interest.

6. ACKNOWLEDGMENTS

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Research Article

Market Behaviour and Economic Analysis of Arrivals and Prices of Potato in Fruit and Vegetable Market Okara (Punjab), Pakistan

Hafiz Saqib Habib[1,2*] and Nasir Mahmood[2]

[1]Price Control and Commodities Management Department, Government of the Punjab, Rawalpindi, Pakistan

[2]Department of Economics and Agricultural Economics, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan

Abstract: The present study was conducted to analyze the behaviour of arrivals and wholesale price of potatoes in the Fruit and Vegetable (F and V) Market of Okara City, Pakistan. Time series data for the last seven years was collected on market arrivals and prices of potatoes from the government department. The Compound Annual Growth Rate (CAGR) was found to be positive (30%) regarding prices, and negative (-7%) for arrivals of potatoes. The variation in prices and arrivals differed across the months. The findings revealed that the highest (211.3%) arrivals variation occurred during the month of November, and the highest (25.89%) price variation took place during the month of February. The results of the Cuddy-Della-Valle Index showed medium instability (24.38 %) in case of prices, and low instability (4.76%) regarding the arrivals. The Karl Pearson Correlation Coefficient method indicated that mostly across the months, there exists a positive relationship between market arrivals and prices of potatoes with the exception of a few months. The study confirmed the highest seasonal arrivals index (338.11%) for the month of February and the highest seasonal price index (136.98%) for the month of November. In order to get maximum benefit from marketing of potatoes, it is suggested that all the concerned stakeholders should look timely and properly at the trend of market prices and market arrivals of potatoes in the F & V markets. Proper management along with market intelligence of arrivals and prices would increase the welfare of farmers, consumers and other stakeholders which involved in the agri-food supply chain of potato.

Keywords: Potato, Market Arrival, Prices, Variation, Instability, Relationship, Farmers, Consumers.

1. INTRODUCTION

The majority of the vegetables are the most important element in the human diet, which play a vital role in supplying vitamins, minerals, fiber, iron, calcium, etc. to the human body. Globally, Potato (*Solanum tuberosum* L.) is one of the largest non-cereal foods and also widely consumed as a tuberous crop. It is globally recognized as fourth most important food crop following rice, wheat and maize crops. The potato comprises various components, including dry matter, edible protein, and edible energy contents, and such composition makes it superior to other vegetables. It plays an important role regarding nutritional balance. Mainly it is consumed as staple food in Pakistan. Further, it is one of important domestic vegetable which is available throughout the year. The estimated production of potato in Pakistan is 76575 thousand quintals [1]. Moreover, production of potato in Punjab province is 74661 thousand quintals [2]. The Punjab province is a major producer of potato in Pakistan. The district Okara is one of highest potato producing district in Punjab. According to the Crop Reporting Service (Government of the Punjab), production of potato in Okara district is 2.21 million tonns in 2023-24, it is sown on an area of 83275 hectares and its yield is 269 (Mounds/Acre) [2] (Table 1).

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* Corresponding Author: Hafiz Saqib Habib <saqibhabib245@gmail.com>

Table 1. Area, production and yields of Potato in Okara District.

Year	Area (Ha)	Production (Tonns)	Yield (Mounds/acre)
2017-18	57997	1400444	261.81
2018-19	58678	1555427	290.48
2019-20	54239	1460663	291.98
2020-21	63547	1595425	254
2021-22	79336	2218140	282.86
2022-23	88259	2255805	258.6
2023-24	83275	2214827	269

Source: Crop Reporting Service, Government of the Punjab [2].

The fluctuation in prices of potato impacts production, consumption and it is one of major factor affecting the income of potato's farmers. Prices of potato show fluctuation both within the year and across the years. Chaudhary *et al*. [3] noted that the stable prices play a key role regarding the determination of farmer's income in agriculture sector. Therefore, it is necessary to consider behaviour of prices regarding growing, storing and selling of the agricultural produce in the agriculture produce markets. Gholap *et al.* [4] emphasized that it is vital to assess the interrelationship between the arrivals of potato and the prices of potato for analyzing price fluctuations and arrival fluctuations in the agricultural produce market. According to the study of Bera *et al*. [5], formulation of effective agricultural price policy regarding stabilization of prices and regulation of supply requires to study interrelationship between farm output prices and arrivals of agricultural commodity. The Thakur *et al*. [6] pointed out that the research studies regarding the price behaviour and market arrivals can assist the agricultural policy makers for devising instructions for ensuring stability in prices and to intimate properly to the producers regarding market conditions so that they make efficient decisions regarding disposal of their agricultural produce at suitable place and time. Although various studies have been undertaken abroad to analyze the behaviour of market arrivals and prices, no such study has been conducted so far in the case of potatoes related to the Okara district (Punjab, Pakistan). In order to fill this gap, the present study was conducted with the aim to properly investigate the extent of relationship between arrivals and market prices of potato so that it can assist concern stakeholders regarding understanding of dynamics of market prices and arrivals of potato in the Agricultural marketing domains of Okara City. This research study would assist not only policy makers but also provide important information to other allied agriculture subsectors for making timely decisions related to potato production and supply chain network for ensuring sustainability. Moreover, consumers would also get critical knowledge regarding when and how much to purchase potato from the F and V Market Okara, it would also assist them to increase their socioeconomic welfare status. The present agricultural marketing study was conducted with reference to F and V Market (Okara), Okara City with the aims to compute the Compound Annual Growth Rate (CAGR), Variability, concerning Instability Value (by using Cuddy-Della- Valle Index), Seasonal indices and extent of relationship between arrivals and market prices of potato.

2. MATERIALS AND METHODS

Monthly data comprising arrivals and prices of potato for Financial Years (FY) 2018 to 2024 was collected from secondary source (Agriculture Marketing Information Service, Government of the Punjab). Furthermore, various analytical techniques were used for estimating growth rate of market arrivals and market prices, extent of variability in prices and arrivals, instability in prices and arrivals, seasonal indices and degree of relationship between market prices and market arrivals of potato in the F and V Market of Okara City.

In order to investigate the trends in market prices and market arrivals of potato concerning F and V Market (Okara) of Okara City, the Compound Annual Growth Rate (CAGR) method is used in this study. This method was also considered by Timilsina and Bhandri [8] regarding study of pricing and arrival behaviour of main trading vegetables in Pokhara market (wholesale) of Nepal. The CAGR calculated between years X and Z (Z-X = N (No. of years between X and Z)) as below:

$$CAGR = \left(\frac{Final\ Value(Year\ Z)}{Initial\ Value(Year\ X)}\right)^{1/N} - 1 \quad (1)$$

For analyzing extent of variability between market arrivals and market prices of potato, the Coefficient

of Variations (C.V) was computed. This method has used by many researchers, including Thakur *et al.* [6] and Chandra *et al*. [7], among others by using the following statistical formula:

$$CV = \frac{Standard\ Deviation}{Mean} \times 100 \qquad (2)$$

The Cuddy-Della-Valle Index is worked out in this study for computing instability level related to prices and market arrivals of potatoes in the F and V market of Okara City. Following formula is used for estimation of C-D-V Index:

$$C - D - V\ Index = CV * \sqrt{(1 - R2)} \qquad (3)$$

Where,
CV = Coefficient of Variation
R^2 = Coefficient of Determination

The Simple Average Approach is used for calculating seasonal prices and arrivals indices of potato. An Index is constructed by dividing each year average price/arrival by the overall seven years average and multiplying by 100.

The statistical measure (Karl Pearson correlation coefficient) is broadly used for computation of degree and direction of relationship existing between the variables which are related in linear form. The relationship between market arrivals and market prices of potato is estimated by using Karl Pearson correlation coefficient for different years from 2018 to 2024. Thakur *et al.* [6] used same methods for studying relationship between market arrivals and concerning prices of potato. The following formula is used:

$$Karl\ Pearson\ correlation\ coefficient\ (r) = \frac{\sum (x - \bar{x})\ (y - \bar{y})}{\sqrt{\sum (x - \bar{x})2 \sum (y - \bar{y})2}} \qquad (4)$$

Where, $\bar{x}$= Mean of variable x,
$\bar{y}$= Mean of variable y

Generally, value of the correlation coefficient (r) ranges from 0 to ± 1. The value of r equal to zero indicates no correlation between two variables, r = 1 depicts perfect positive correlation and r = -1 revealed perfect negative correlation between two variables.

3. RESULTS AND DISCUSSION

It has been observed by many researchers that there is a widespread fluctuation in the output of potato because of its perishability and seasonality nature. Resultantly, such variations lead to cause fluctuations in the prices of potato. Saha *et al.* [9] observed that despite the fact that there are various factors that are responsible for creating such variations in the prices of potatoes, market arrival plays an important role in determining prices in the agricultural produce markets. Therefore, it is important to study the relationship of prices and market arrival for ensuring better understanding of agricultural marketing system concerning to specific crop (potato).

3.1. Growth Rate of Market Prices and Arrivals of Potato

Over the span of seven years there is a positive CAGR value (30%) in the prices of potato. It is mainly due to rising population, increase in awareness regarding health and market inflation. However, there is a negative CAGR value (-7%) in case of arrivals of potato in F and V market of Okara City. The reason could be due to access of local producers to other F and V Markets of Pakistan, the supply to F and V market of Okara City has shown negative trend because such farmers received higher profit margin by selling potato in other F and V Markets. The findings are in consistent with the study of Chandra *et al.* [7] regarding arrivals of potato in Nagpur Agriculture Produce Market.

3.2. Forecasting of Arrivals and Prices of Potato

It is important to forecast the future trend of arrivals and prices to inform concerned stakeholders in making decisions regarding input purchase and allocation of land for growing of suitable crop. Figure 1 shows the arrival data from 2018 to 2024 and a linear trend is forecasted for the future time period (2025 to 2027). These results confer that arrivals trend shows decreasing behaviour. Figure 2 shows a comparative trend of price from 2018 to 2024, these results show an increasing trend; the price forecast for 2025 to 2027 shows a linear increase in the price.

3.3. Average Monthly Variability in Market Arrivals and Prices of Potato in F & V Market Okara

Variability on monthly basis regarding the market

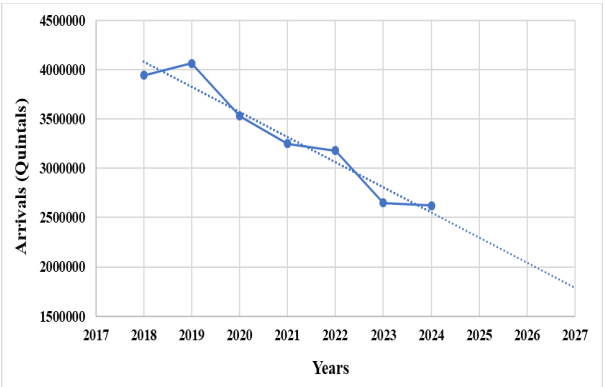

Fig 1. The year wise arrivals of potato (symbols and solid line show data used in the study, while dotted line is the linear fit for future trend).

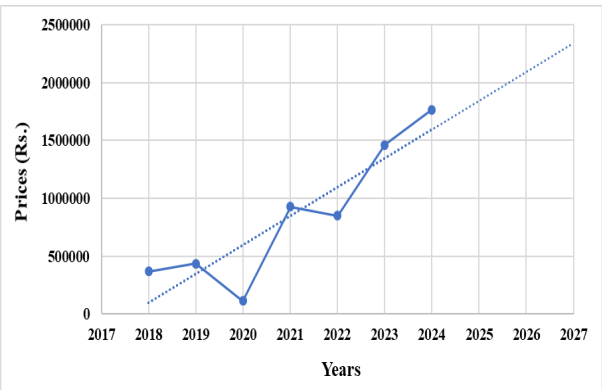

Fig. 2. The year wise prices of potato (symbols and solid line show data used in the study, while dotted line is the linear fit for future trend).

arrivals and prices of potato is shown in Table 2. There is a significant variation in arrival of potato across the months. Highest monthly mean arrivals were observed during February (225770 quintals) and lowest monthly mean arrivals were noted during August (269.67 quintals) over the span of seven years. Likewise, prices also show variations from month to month. The Highest mean monthly prices were noticed during November (Rs.26435 per quintal) and lowest mean monthly prices were noticed during February (Rs.11260.240 per quintal). Similar pattern was observed by Singh *et al.* [10] in Agra Market (India) who concluded that there were highest monthly mean prices in November and it was noticed lowest level during February. Moreover, highest considerable variation in arrivals relative to the mean value was observed during November (211.3 percent) and value of lowest variation in arrivals relative to the mean value was noticed during January (21.5 percent). These results are consistent with the findings of Thakur *et al.* [6], who stated that there were the highest variations in arrivals relative to the mean during the month of November and the lowest variation in arrivals relative to the mean during the months of January and February in the Delhi Market of India. As far as variations in prices were concerned, highest substantial variation in prices relative to the mean were observed in the month of February (25.89 percent) and lowest variations noticed in the month of October (12.77 percent).

Table 2. Average monthly variability in arrivals and prices of Potato in Okara Market.

Months (FY-2018 to 2024)	Arrivals		Prices	
	Mean (Quintals)	C.V %	Mean (Rs.)	C.V %
Jan	154816.5	21.5	13925.45	14.42
Feb	225770	24	11260.24	25.89
Mar	180016.7	25.86	13521.94	18.49
Apr	94277.07	35.71	15817.4	20.26
May	25623.71	64.45	18392.74	22.29
Jun	851.43	101.7	19598.4	19.18
Jul	293.09	41.43	20058.68	17.76
Aug	269.67	29.32	20278.06	21.83
Sep	327.8	20.17	21500.83	12.86
Oct	395.9	21.64	22140.32	12.77
Nov	2471.86	211.3	26435	16.66
Dec	82198.84	47.72	24592.74	18.94

Source: Author's own calculations.

3.4. Measurement of Instability in Arrivals and Prices of Potato by using C-D-V- Index

The level of instability related to arrivals and prices in F and V Market Okara was estimated by using C-D-V-Index. Table 3 shows that there is a low instability (4.76 %) in case of arrivals of potato over the period of seven years in the Okara F and V Market. Moderate instability was observed in case of prices of potato over the seven years because of various factors which are linked with formulation of prices in the market. Such moderate instability had an unfavorable impact on farmers and consumers.

3.5. Seasonality of Market Arrivals and Prices of Potato

The monthly seasonal indices of market arrivals and market prices of potato concerning F and V Market (Okara) for the period of last seven years were shown in Table 4. These findings indicated that market arrivals of potato observed as low from the month of June to November and it is in consistent with the findings of Singh *et al.* [10]. The minimum arrival index (0.43) was noted in August. However, increase in arrival index were observed from November to May. The arrival index (maximum) was noticed during the month of February reaching value of 338.11. These results of arrivals are in agreement with the findings of Chandra *et al.* [7] and Patel *et al.* [11] who stated that pattern of market arrivals of potato was maximum during January to March in Nagpur and Deesa Markets. The price index (56.40) was lowest during February and highest price index was noticed in November and these findings were consistent with the results of Singh *et al.* [10], Chandran and Pandey [12], Dhakre and Bhattachrya [13], and Noonari *et al.* [14] with reference to their studies in Delhi (India), Agra (India) and Hyderabad (district of Pakistan) Markets.

3.6. Relationship between Arrivals and Prices of Potato in F and V Market (Okara)

The degree and direction of relationship is analyzed between market arrivals and market prices of potato in F and V Market (Okara) over the period of seven years by using Karl Pearson correlation coefficient. The monthly correlation coefficients were shown in Table 5 and mostly the value of correlation coefficient is positive because of various reasons including off season supply of potato to the market and availability of cold storages, among others. This

Table 3. Instability index of potato in F and V Market (Okara) over the period of 2018 to 2024.

Variable	C.V	Instability Index (%)	Inference
Arrival	17.18	4.76	Low Instability
Price	51.39	24.38	Moderate Instability

Source: Author's own calculations.

Table 4. Seasonal monthly indices of market arrivals and prices of Potato in Okara market.

Months	Arrival Index (%)	Price Index (%)
January	247.84	74.56
February	338.11	56.40
March	288.18	72.40
April	146.05	81.96
May	41.02	98.48
June	1.31	101.55
July	0.46	107.40
August	0.43	108.58
September	0.50	111.41
October	0.63	118.55
November	3.82	136.98
December	131.59	131.68

Source: Author's own calculations.

Table 5. Correlation coefficients between market arrivals and market prices of potato.

Month	Potato
January	-0.11
February	0.58
March	0.40
April	-0.19
May	0.21
June	0.13
July	0.23
August	0.19
September	0.18
October	-0.009
November	0.41
December	-0.40

Source: Author's own calculations.

result is in consistent with the findings of Thakur *et al.* [6]. Moreover, the study of Areef *et al.* [15] also showed parallel results in which most of the months revealed positive correlation coefficients and value of coefficient of correlation is also highest during the month of February (harvesting season) as far as their study concerned with the Bangalore Market. On the other hand, there were inverse relationship between market arrivals and prices during the month of January, April, October and December regarding the present study in F and V market Okara.

4. CONCLUSIONS

This study has examined the behaviour and economic analysis of market arrivals and market prices of potatoes in the F and V market (Okara) of Punjab province of Pakistan over the period of seven years. The findings show that there is an increase in growth rate in the prices and reduction in the growth rate in the market arrivals of potato. The variability in the market arrivals was highest in the month of November, and the variability in the case of prices was highest in the month of February. The mean monthly market arrival of potato significantly increased for January and attained highest value for the month of February (225770 quintals) and resultantly lowest mean price was noticed during the month February. Moreover, the C-D-V index indicates low instability in case of arrivals and moderate instability as far as prices were concerned. The seasonal indices indicate that arrival index was low during the period of June to November and Price index shows low value during December to May which is consistent with theoretical economic approach. Most of the months showed positive relationship between market arrivals and market prices of potato. It is suggested that all the stakeholders engaged in the supply chain of potato must collaborate timely and efficiently with each other in transparent manners so that overall welfare of all the concern stakeholders would be increased in an efficient way. The effective management of market arrivals and prices of potato would increase the welfare for farmers, consumers and for all other stakeholders which are engaged in agri-food supply chain network of potato. There is a need to study consumer demand of potato related to their preferences.

5. CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

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Research Article

Nutritional Evaluation of Gum Arabic and Its Food Products Locally Prepared in Tharparkar, Sindh, Pakistan

Pawan Kumar[1], Aijaz Hussain Soomro[1], Asadullah Marri[1], Omer Mukhtar Tarar[2], Nida Shaikh[1], Asif Irshad[3*], Allah Bux Baloch[1], and Muhammad Dawood[1]

[1]Institute of Food Sciences and Technology, Sindh Agriculture University, Tandojam, Hyderabad, Pakistan

[2]Food and Marine Resources Research Centre, Pakistan Council of Scientific & Industrial Research (PCSIR), Laboratories Complex, Karachi, 75280, Pakistan

[3]Department of Food Preservation, Technical Wing of Colleges, Higher & Technical Education Department, Balochistan, Pakistan

Abstract: Gum Arabic is an edible, dried, gummy exudate extracted from the stems and branches of *Acacia Senegal*. It is commonly used in the pharmaceutical and food industries as an emulsifier and stabilizer agent. The present study assessed the nutritional composition of gum Arabic (T_1) and its food products, namely T_2 (Khorak) and T_3 (Gond Pak), which were locally prepared in Tharparkar, Sindh, Pakistan. Gum Arabic was sourced from the local tree *Acacia Senegal,* whereas its food products, i.e., Khorak and Gond Pak, were prepared and evaluated for proximate composition, amino acids profiling, minerals, and vitamins quantification. The observed results showed significant differences ($p \leq 0.05$) among treatments. The T_1 exhibited higher values for ash (3.17%), dietary fiber (66.10%), titratable acidity (1.5%), valine (0.30 mg/g), serine (0.68 mg/g), potassium (254.22 mg/100g), sodium (118.55 mg/100g), calcium (7976.30 mg/kg), magnesium (2061.5 mg/kg), iron (76.57 mg/kg), and thiamine (7.74 mg/100g). T_2 showed higher pH (5.47), moisture (11.61%), carbohydrate (39.25%), energy value (308.65 kcal/100g), methionine (0.02 mg/g), isoleucine (0.16 mg/g), leucine (0.68 mg/g), tyrosine (0.39 mg/g), lysine (3.35 mg/g), vitamin A (141.33 IU/100g), vitamin C (4.65 mg/100g), niacin (26.25 mg/100g), and pyridoxine (0.06 mg/100g). T_3 had higher protein (12.29%), crude fat (15.99%), aspartic acid (1.66 mg/g), threonine (0.533 mg/g), glutamic acid (4.43 mg/g), proline (0.079 mg/g), glycine (0.446 mg/g), alanine (0.443 mg/g), phenylalanine (0.54 mg/g), histidine (0.20 mg/g), tryptophan (0.92 mg/g), riboflavin (0.71 mg/100g), folate (21.97 mg/100g), and cyanocobalamin (1.17 mg/100g). Cysteine, arginine, vitamin D, E, pantothenic acid, and biotin were not detected in any treatments. The study concludes that gum Arabic and its food products are highly nutritious, suggesting their suitability for consumption in regions with prevalent malnutrition.

Keywords: Gum Arabic, Traditional Foods, Nutritional Composition, Amino Acids, Malnutrition, Tharparkar Sindh.

1. INTRODUCTION

Gum Arabic, as defined by the FAO/WHO Joint Expert Committee for Food Additives (JECFA), is a dried exudate that is extracted from the stems of *A. Senegal* Willdenow or closely related species of Acacia (family Leguminosae) [1]. Gum Arabic is obtained from the hardened secretions found on the injured stems and branches of the *Acacia Senegal* trees, which are then harvested as air-dried globules. It is a complex, branched polysaccharide. It exists as a weak acidic salt or as a neutral polymer that dissolves in water. Its low-solution viscosity, non-digestibility, and safety make it a popular emulsifier, stabilizer, and thickening agent in industrial food manufacturing. In addition to having good solubility, low viscosity, a good binding effect, and the capacity to create films, it also emulsifies well and retains volatile components in food products. In confections, it is used to stop

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* Corresponding Author: Asif Irshad <asifirshadbaloch@gmail.com>

sugar from crystallizing. It is used as a glaze in candies. It finds larger employment in flavor encapsulation and is utilized in bakery, dairy, and soft and alcoholic items. It acts as a clouding agent or flavoring stabilizer in alcoholic beverages [2, 3]. It is certified as a safe dietary fiber by the Food and Drug Administration (FDA) [4, 5]. It is one of the oldest and most significant industrial gums in the world, consisting of proteins, minerals, and arabinogalactan polysaccharides [6, 7]. In Pakistan, agriculture is a climate-sensitive sector. Most of the regions are facing severe water shortages along with changes in rainfall patterns, specifically in the least developed area, i.e., Tharparkar [8]. Tharparkar is an arid region located in the southeastern part of the Sindh province of Pakistan and is experiencing drought as a regular phenomenon [9]. The region of Tharparkar is blessed with a wide range of drought-tolerant crops, including *Acacias, i.e., Acacia Senegal*. The gum obtained from *Acacia Senegal* is abundantly produced in the area. The locals use it extensively for a variety of food-related and medicinal purposes. The food-related uses of gum Arabic are common among the people of Tharparkar. The people of Tharparkar prepare some traditional foods from gum Arabic, and they believe that this natural substance exerts beneficial effects on health if consumed through food products. Traditional foods are generally a combination of energetic staples with other available ingredients [10]. Traditional recipes for intermixing cultures, mixed habitation, and resources are mostly prepared from local plants and can play a vital role in community nutrition. Most people in Tharparkar use gum Arabic to prepare some traditional foods, such as Khorak and Gond Pak. The present study was conducted to evaluate the nutritional composition of gum Arabic and its locally prepared food products, i.e., Khorak and Gond Pak from Tharparkar, Sindh, Pakistan, by analyzing their proximate composition, amino acid profile, mineral, and vitamin contents, to determine their potential contribution towards improving nutrition and combating malnutrition in the region.

2. MATERIALS AND METHODS

2.1. Raw Materials

Gum Arabic was gathered from the native trees of *Acacia Senegal* of the Tharparkar district (24.8777° N, 70.2408° E). The samples of gum Arabic were kept in a glass jar and transported to the Laboratories of the Institute of Food Sciences and Technology, Sindh Agriculture University, Tandojam. For the preparation of Khorak and Gond Pak, the materials (i.e., sugar, ghee, butter, all-purpose wheat flour, coconut powder, small cardamom, almond, and black raisin) were purchased from the local market of Hyderabad city and were used to prepare the study samples.

2.2. Preparation of Samples

The gum Arabic was sorted based on its crystal clarity, washed to remove dust, air dried, ground with an electric grinder, screened using an 80-mesh sieve, collected in a clean and sterilized airtight glass jar, properly labeled, and stored until further use by following the method of Abel *et al.* [11] with minor modifications.

2.3. Preparation of Khorak and Gond Pak

The traditional food, Khorak, was prepared as per the method used by the local women of Tharparkar, Sindh. Table 1 shows the list of ingredients and their quantity used in making Khorak. For preparing Khorak, the gum Arabic was roasted in butter and cooled. On the other side, wheat flour was also roasted in butter and set aside to cool. After that, water, butter, and sugar were added to a pan and heated on a low flame to prepare a thick concentrate. Finally, roasted gum Arabic, roasted flour, ground almond, black raisin, and small cardamom were added, and the content was mixed properly. Khorak was kept in airtight glass jars, labeled, and kept under refrigeration till analysis.

Table 2 represents the list of ingredients and their quantity for preparing Gond Pak. The Gond Pak was prepared by roasting gum Arabic in butter.

Table 1. List of ingredients and their quantity used in Khorak preparation.

Ingredients	Quantity (g)
Wheat flour	200
Gum Arabic	200
Butter	200
Sugar	300
Almond	50
Black raisin	20
Small cardamom	10

Table 2. List of ingredients and their quantity used in Gond Pak preparation.

Ingredients	Quantity
Gum Arabic	400 gm
Butter	150 gm
Water	100 mL
Sugar	300 gm
Coconut powder	150 gm
Almond	50 gm
Poppy seeds	10 gm

After that, a pan containing water, butter, and sugar was placed on a low flame to prepare a concentrate. The roasted gum Arabic, coconut powder, and poppy seeds were added, and the content was mixed properly. After mixing, the content was poured into a flat plate, garnished with shredded almonds and peanuts, allowed to cool, and set. Finally, the set dessert (Gond Pak) was cut into uniform pieces using a stainless-steel knife and kept in airtight glass jars, labeled, and kept under refrigeration temperature till further analysis.

2.4. Nutritional Analysis of Gum Arabic, Khorak, and Gond Pak

2.4.1. Proximate analysis

A pH meter (Model HI, Hanna Instruments, Italy) was used to measure the pH value of the gum Arabic and its food products according to the method of AOAC [12]. Crude protein (%), moisture (%), ash (%), crude fat (%), dietary fiber (%), and titratable acidity (%) were analyzed by following the standard methods of AOAC [12].

A difference method was used to determine the samples' available carbohydrate content. The percentage of moisture, protein, fat, ash, crude, and dietary fiber was subtracted to determine the amount of carbohydrates by following the Hart and Fisher [13] method. This value was treated as a carbohydrate and was calculated using the following equation.

Carbohydrate (NFE g%) = 100 − (protein+ lipid + moisture +ash + fiber)

Energy or calorific value was calculated based on the method outlined by Paul and Southgate [14], using energy conversion factors of protein, fat, and carbohydrates in the foods. The calculation was performed using the standard conversion factors: 4 kcal/g for protein, 9 kcal/g for fat, and 3.75 kcal/g for carbohydrates.

2.4.2. Amino acid analysis

Acid hydrolysis was performed according to the procedure outlined in AOAC [12] through the Ion Exchange Chromatographic method. Food samples (100 mg) were taken in separate digestive tubes with the addition of HCl (6N; 5 mL) carrying 0.1% Phenol and kept for 18-24 hours at 110 °C. The hydrolyzed samples were dried by vacuum evaporation at 60 °C in a rotary evaporator. Then, it was rinsed with 20 mL of water and re-evaporated. The washing and evaporating process was carried out twice. 50 mL of sodium citrate buffer was used to make up the final volume. Before injection, samples were filtered through a 0.22-micron filter, with a 20 µL volume being introduced into the amino acid analyzer. The analysis was conducted using an application data book and a Shim-Pack Amino-Na column (4.6 mm internal diameter by 100 mm length) as part of the Shimadzu HPLC amino acid analysis system. A fluorescence detector was utilized with an excitation wavelength of 350 nm and an emission wavelength of 450 nm. Peaks were identified through a post-column derivatization reaction, where eluted amino acids reacted with o-phthalimide (OPA).

2.4.3. Mineral analysis

Sodium, Potassium, Calcium, and Magnesium were analyzed following the standard methods outlined in AOAC [12]. Dry ashing was used in the muffle furnace to remove the organic matrix. Atomic absorption spectrophotometry was used to test the analyte after the remaining ash was dissolved in diluted acid. To determine Ca and Mg only, 0.1% (w/v) La was included in each standard and test solution's final dilution using $LaCl_3$ solution. To determine Na and K, 0.5% (w/v) Cs (0.04M) was made by adding CsCl solution to the final dilution of each reference and test solution. Blanks had been prepared and carried throughout the process. For each mineral to be identified, a calibration curve showing concentration vs absorbance was prepared. Flame parameters were followed by the directions of the instrument manufacturer's instructions.

According to the instrument instruction manual, calibration solutions were created to cover the linear range of the calibration curve. Similar procedures were used to assay the samples. Each mineral's concentration was determined from its calibration curve, and the concentration in the test sample was calculated while taking dilutions and test portion size into account.

2.4.4. Vitamins analysis

2.4.4.1. Fat-soluble vitamins β-carotene

HPLC was used to estimate the β-carotene using the official method outlined in AOAC [12]. The sample was homogenized and saponified in an ethanolic potassium hydroxide solution, and the β-carotene that was released was completely extracted using organic solvents. Using reversed-phase HPLC, retinol content was separated from a portion of the extract. Quantification was done by the β-carotene standard.

2.4.4.2. Vitamin A, D, and E

The samples were prepared using the methodology outlined by Kirk and Sawyer [15]. Then, the liquid chromatographic analysis was conducted per the guidelines established by Agilent Technology [16]. First, weigh 5.0 g of finely ground and homogenized samples and transfer them into a 100 mL round-bottom flask. Added 25 mL of absolute ethanol to dissolve the sample matrix, and after dissolving, added 10 mL of 50% potassium hydroxide (KOH) solution along with 0.5 g pyrogallol. The mixture was subjected to saponification under reflux conditions at 70 - 80 °C for 30 minutes with continuous stirring. After rapid cooling, the saponified mixture was transferred into a separating funnel. Vitamins A, D, and E were extracted with diethyl ether (3 × 25 mL). The ether extracts were washed with distilled water until a neutral pH was achieved. Anhydrous sodium sulphate was used to dry the mixture of extracts, and then a lower pressure was used to evaporate them completely. The dried residue was reconstituted in 5 mL of methanol. A 0.45 µm membrane filter was used to filter the solution before it was placed in amber HPLC vials for examination. Stock standard solutions of Vitamins A, D, and E were prepared in methanol at a concentration of 1 mg/mL. Working standard solutions were prepared by serial dilution to obtain concentrations of 5, 10, 20, 40, and 80 µg/mL. Each standard solution was injected into the HPLC, and the peak area versus concentration was plotted to create a standard calibration curve. Vitamin concentrations in the samples were calculated using the regression equations obtained from the standard calibration curves.

2.4.4.3. Water-soluble vitamins

Following the procedure outlined by Kirk and Sawyer [15], water-soluble vitamins were subjected to a liquid chromatographic analysis under the guidelines established by Agilent Technology [16]. First, weigh 10.0 g of the sample and add the sample to 90 mL of distilled water, and homogenize for 2 minutes using a high-speed homogenizer to produce a 10% (w/v) homogenate. Transferred 10.0 mL of the homogenized suspension to a 250 mL conical flask. To the 10.0 mL homogenate aliquot, 50.0 mL of 5 N HCl was added, capped the flask, and placed in a boiling-water bath at 100 ± 2 °C.

After 30 minutes, removed the flask and allowed it to cool to room temperature (20–25 °C). Adjusted the pH to 4.0–4.5 using 2.5 M sodium acetate buffer. Added 5.0 mL of a 10% (w/v) Taka-diastase (α-amylase) solution to the digest. Incubated the mixture at 45 ± 2 °C for 3.0 hours in a shaking/temperature-controlled water bath (set to 100 rpm agitation for mixing). After the 3-hour incubation, the digest was cooled to room temperature and filtered through Whatman No.1 filter paper to remove coarse solids. Transferred the filtrate to a 100 mL volumetric flask and made up to the mark with distilled water (final volume = 100 mL). Before HPLC injection, a portion of the extract was filtered through a 0.2 µm syringe filter into HPLC vials.

Prepared the primary stock standard solutions by accurately weighing reference standards and dissolving them in deionized water. From each stock, a working standard was prepared by pipetting 1.0 mL of stock into a 50.0 mL volumetric flask and diluting to the mark with deionized water. From the working standard, a five-point calibration series by serial dilution to cover the expected sample concentration range. Plotting a standard curve between concentrations and peak area, followed by linear least squares regression, yielded a regression equation that was used to estimate vitamin content in a variety of samples.

2.4.4.4. Vitamin C

The total vitamin C content, identified as ascorbic acid, was measured using LC-UV. A phosphate buffer (pH 2.5) served as the mobile phase for separation on a C_{18} reverse-phase column, with detection occurring at 248 nm. An automated LC system featuring a pump for continuous delivery at 0.5–1.0 mL/min and a precision injection device for 50 µL. A UV detector with a stable baseline was set at 248 nm. The UV-VIS detector was in place. Suitable 5 µm monomeric silica-based C18 reverse phase column (nominally 150 × 4 mm) [17].

2.5. Statistical Analysis

A total of three replications were studied for all tests. The collected data from the study were tabulated and analyzed using the statistical procedure of analysis of variance (ANOVA). The method outlined by Gomez and Gomez [18] was used to further compute significant differences in the mean using the least significant difference (LSD) at a 0.05% level of probability.

3. RESULTS

3.1. Proximate Composition

Gum Arabic, Khorak, and Gond Pak significantly differed ($p \leq 0.05$) in their average proximate composition, as shown in Table 3. Gum Arabic had the lowest pH, protein, moisture, fat, carbohydrate, and energy value among the three, while Khorak had the highest pH, moisture, carbohydrate, and energy value. Gond Pak had the highest protein and crude fat.

3.2. Amino Acid Analysis

Gum Arabic and its food products, Khorak and Gond Pak, displayed significant differences ($P \leq 0.05$) in their amino acid profiles as shown in Table 4. Gond Pak had the highest levels of most amino acids analyzed, including aspartic acid, threonine, glutamic acid, proline, glycine, alanine, phenylalanine, histidine, and tryptophan. Methionine, isoleucine, leucine, tyrosine, and lysine were highest in Khorak. Serine and valine were highest in gum Arabic, while cysteine and arginine were not detected in any of the samples.

3.3. Mineral Analysis

The mineral content of gum Arabic was significantly higher ($P \leq 0.05$) than that of Khorak and Gond Pak across all measured elements. Potassium, sodium, calcium, magnesium, and iron were all highest in gum Arabic, followed by Khorak and Gond Pak, as shown in Table 5. These findings highlight the distinct mineral profiles of these three food products, with gum Arabic offering the most concentrated source of these essential minerals.

3.4. Vitamins Analysis

Gum Arabic, Khorak, and Gond Pak were significantly different ($P \leq 0.05$) in their vitamin content. Khorak had the highest vitamin A, C, niacin, and pyridoxine, while Gond Pak had the

Table 3. Average proximate Content/ Composition of gum Arabic and its food products.

Content/Composition	Treatments			LSD (0.05)	SE ±
	T_1	T_2	T_3		
pH Value	4.92 ± 0.005c	5.47 ± 0.01a	5.16 ± 0.005b	0.0007557	0.0002722
Crude Protein%	3.41 ± 0.09c	7.96 ± 0.27b	12.29 ± 0.18a	0.5261	0.1895
Moisture%	8.30 ± 0.03c	11.61 ± 0.11a	8.95 ± 0.27b	0.2857	0.1029
Ash%	3.17 ± 0.02a	1.02 ± 0.03c	1.23 ± 0.11b	0.1867	0.0672
Crude Fat%	0.35 ± 0.075b	15.49 ± 0.87a	15.99 ± 0.29a	1.2794	0.4608
Dietary Fiber%	66.10 ± 0.43a	24.66 ± 0.40c	37.30 ± 0.44b	1.1261	0.4056
Carbohydrate%	18.66 ± 0.40c	39.25 ± 1.47a	24.22 ± 1.09b	2.9028	1.0455
Titratable Acidity%	1.50 ± 0a	0.14 ± 0.01b	0.13 ± 0.005b	0.0151	0.0005443
Energy value (kcal/100g)	82.14 ± 1.87c	308.65 ± 3.7a	277.87 ± 2.9b	7.0104	2.5250

T_1 = Gum Arabic, T_2 = Khorak, T_3 = Gond Pak, LSD = Least Significant Difference, SE = Standard Error

Table 4. Average amino acid content of gum Arabic and its food products.

Amino acid content (mg/g)	Treatments			LSD (0.05)	SE ±
	T_1	T_2	T_3		
Aspartic acid	0.698 ± 0.00b	0.37 ± 0.01c	1.663 ± 0.00a	0.0122	0.0004384
Threonine	0.39 ± 0.001b	0.14 ± 0.01c	0.533 ± 0.001a	0.0122	0.0004384
Serine	0.68 ± 0.001a	ND	ND	0.0001308	0.004714
Glutamic acid	0.578 ± 0.001c	1.39 ± 0.015b	4.43 ± 0.001a	0.0192	0.0006909
Proline	0.041 ± 0.00c	0.06 ± 0.001b	0.079 ± 0.00a	0.0122	0.0004384
Glycine	0.188 ± 0.00b	0.12 ± 0.01c	0.446 ± 0.00a	0.0122	0.0004384
Alanine	0.13 ± 0.00b	0.14 ± 0.01b	0.443 ± 0.00a	0.0122	0.0004384
Valine	0.303 ± 0.01a	ND	0.302 ± 0.00a	0.0001603	0.0005774
Methionine	ND	0.02 ± 0.01a	ND	0.0131	0.0003714
Isoleucine	ND	0.16 ± 0.01a	0.117 ± 0.00b	0.0125	0.0004497
Leucine	ND	0.68 ± 0.01a	0.03 ± 0.001b	0.0125	0.0004497
Tyrosine	ND	0.39 ± 0.01a	0.199 ± 0.00b	0.0125	0.0004497
Phenylalanine	ND	0.43 ± 0.01b	0.547 ± 0.00a	0.0125	0.0004497
Histidine	0.163 ± 0.001b	0.05 ± 0.01c	0.204 ± 0.00a	0.0122	0.0004384
Tryptophan	ND	0.32 ± 0.01b	0.921 ± 0.00a	0.0125	0.0004497
Lysine	ND	3.35 ± 0.01a	0.099 ± 0.00b	0.0125	0.0004497
Cysteine	ND	ND	ND	-	-
Arginine	ND	ND	ND	-	-

T_1 = Gum Arabic, T_2 = Khorak, T_3 = Gond Pak, LSD = Least Significant Difference, SE = Standard Error, ND = Not detected

Table 5. Average mineral Content/ Composition of gum Arabic and its food products.

Mineral content	Treatments			LSD (0.05)	SE ±
	T_1	T_2	T_3		
Potassium (mg/100g)	254.22 ± 0.5a	112.49 ± 0.1b	84.77 ± 0.33c	1.1241	0.4086
Sodium (mg/100g)	118.55 ± 0.2a	39.35 ± 0.06b	39.53 ± 0.15b	0.5198	0.1872
Calcium (mg/kg)	7976.3 ± 12a	1485.7 ± 9.5c	1680.7 ± 13b	142.66	51.383
Magnesium (mg/kg)	2601.5 ± 39a	1238.1 ± 7.9b	1200.5 ± 9b	39.900	14.371
Iron (mg/kg)	76.57 ± 1.15a	46.551 ± 0.2b	44.659 ± 0.3c	1.0830	0.3901

T_1 = Gum Arabic, T_2 = Khorak, T_3 = Gond Pak, LSD = Least Significant Difference, SE = Standard Error

highest riboflavin, folate, and cyanocobalamin. Gum Arabic had the highest thiamine, while vitamin D, E, pantothenic acid, and biotin were not detected in any of the treatments, as shown in Table 6. Overall, Khorak appears to be the most vitamin-rich of the three, followed by Gond Pak and Gum Arabic.

4. DISCUSSION

The kingdom Plantae boasts over 1350 species [19], with *Acacia Senegal* and *Acacia Seyal* being crucial for gum Arabic production. This ancient gum, derived from Acacia trees, serves as a stabilizer, emulsifier, and thickening agent in various food products. The present study analyzed gum Arabic from Acacia trees in the Tharparker district of Sindh, Pakistan, examining its chemical composition and nutritional value, along with traditional recipes from gum Arabic, i.e., Khorak and Gond Pak.

The therapeutic uses of Acacia plants, particularly gum Arabic, have been extensively documented in folklore and tradition [20]. William

Table 6. Average vitamin content of gum Arabic and its food products.

Vitamin content	Treatments			LSD (0.05)	SE ±
	T_1	T_2	T_3		
Vitamin A (IU/100g)	78.33 ± 0.75b	141.33 ± 1.5a	12.67 ± 1.52c	1.2766	3.5443
Vitamin D (IU/100g)	ND	ND	ND	-	-
Vitamin E (IU/100g)	ND	ND	ND	-	-
Vitamin C (mg/100g)	0.72 ± 0.005c	4.65 ± 0.000a	1.12 ± 0.003b	0.0008861	0.0003192
Thiamine (mg/100g)	7.74 ± 0.005a	4.16 ± 0.005b	2.06 ± 0.005c	0.0007557	0.0002722
Riboflavin (mg/100g)	0.27 ± 0.05c	0.32 ± 0.005b	0.71 ± 0.05a	0.0007557	0.0002722
Niacin (mg/100g)	2.47 ± 0.05c	26.25 ± 0.01a	4.83 ± 0.01b	0.0151	0.0005443
Pantothenic acid (mg/100g)	ND	ND	ND	-	-
Pyridoxine (mg/100g)	ND	0.06 ± 0.005a	0.01 ± 0b	0.0007557	0.0003722
Biotin (mg/100g)	ND	ND	ND	-	-
Folate (mg/100g)	8.58 ± 0.05b	4.20 ± 0c	21.97 ± 0.01a	0.0131	0.0004717
Cyanocobalamin (mg/100g)	0.67 ± 0.05b	0.61 ± 0.005c	1.17 ± 0.05a	0.014	0.034

T_1 = Gum Arabic, T_2 = Khorak, T_3 = Gond Pak, LSD = Least Significant Difference, SE = Standard Error, ND = Not detected

and Phillips [21] noted that gum Arabic is water-soluble, with a pH value around 4.5. However, in the present study, gum Arabic's pH remained at 4.92, while Khorak and Gond Pak had pH values of 5.47 and 5.16, respectively. Nour [22] reported similar pH values for gum Arabic, i.e., 4.35-4.64. A relatively greater pH value of traditional recipes, i.e., Khorak and Gond Pak, might be associated with the ingredients used to prepare these dishes. Titratable acidity, an indicator of organic acid [23], was 1.5%, 0.14%, and 0.13% in gum Arabic, Khorak, and Gond Pak, respectively. This indicates a substantial level of organic acid content in these commodities. Moisture content in gum Arabic was 8.30%, differing from El-Kheir *et al.* [24], i.e., 10 to 16.15%. Khorak and Gond Pak had moisture contents of 11.61% and 8.95%, respectively. The fact behind the low content of moisture in Khorak and Gond Pak is linked with the minimal use of extraneous water during their preparation. Ash content, reflecting total mineral content, was 3.17%, 1.02%, and 1.23% in gum Arabic, Khorak, and Gond Pak, respectively. In connection with Ali and Daffalla [25], the ash content in gum Arabic was recorded to be 2.5%. However, the present findings for ash content align with the findings of Lelon *et al.* [26], which suggest that gum Arabic from Acacia Senegal had ash content ranging from 2.72 to 3.16%. The protein content in gum Arabic, Khorak, and Gond Pak was 3.41%, 7.96%, and 12.29%, with higher protein content in traditional recipes due to protein-rich ingredients (i.e., almond, coconut, poppy seeds, etc.). In a similar study, Ali and Daffalla [25] found the protein content of gum Arabic to be 2.2%. Gum Arabic had a negligible fat content (0.35%), while Khorak (15.49%) and Gond Pak (15.99%) exhibited higher fat content. The fat content of gum Arabic is less; however, it has great potential to disperse fat micelles in a food product since it is an emulsifier [27]. Dietary fiber content was 66.10% in gum Arabic, 24.66% in Khorak, and 37.30% in Gond Pak. Dietary fiber is crucial, with some types acting as prebiotics, benefiting gut bacteria and overall health [28]. Many researchers, including Niamah *et al.* [29] and Talib *et al.* [30], determined that gum Arabic, rich in dietary fiber, behaves as a prebiotic in yogurt, highlighting its substantial fiber content. Carbohydrate levels were highest in Khorak (39.25%), followed by Gond Pak (24.22%) and Gum Arabic (18.66%). Carbohydrates are the main constituents of gum Arabic. According to Dauqan and Abdullah [31], gum Arabic's composition changes with climate and soil, but it's always a complex sugar molecule (polysaccharide) with calcium, magnesium, and potassium. Traditional recipes, with their added table sugar, have even higher carbohydrate content. The energy values (kcal/100g) were 308.65, 277.87, and 82.14 for Khorak, Gond Pak, and gum Arabic, respectively, indicating higher nutritional value

in traditional recipes made from gum Arabic. The higher energy value of the traditional recipes made from gum Arabic shows their nutritional value.

Amino acids are needed for the survival, development, growth, and reproduction of all organisms [32]. The polysaccharide in gum Arabic is associated with a certain protein fraction in which hydroxyproline and proline are the chief types of amino acids. In the present study, some essential and non-essential amino acids were analyzed in gum Arabic, Khorak, and Gond Pak, revealing significant amino acid content. Among different amino acids, serine was recovered considerably in gum Arabic, i.e., 0.68 mg/g in comparison to Khorak and Gond Pak. Khorak displayed higher methionine content (0.02 mg/g), while Gond Pak contained significant levels of aspartic acid (1.663 mg/g), threonine (0.533 mg/g), glutamic acid (4.435 mg/g), proline (0.079 mg/g), glycine (0.446 mg/g), histidine (0.204 mg/g), and alanine (0.443 mg/g). Valine content was similar in both gum Arabic and Gond Pak (0.303 mg/g). Gum Arabic lacked detectable amounts of methionine, isoleucine, leucine, tyrosine, phenylalanine, tryptophan, lysine, cysteine, and arginine. In contrast, Khorak and Gond Pak contained reasonable quantities of these amino acids. Gum Arabic revealed the presence of valine and histidine among essential amino acids, emphasizing their importance in human protein formation. In a similar study, Nour [22] found that *Acacia laeta* gum had the highest amino acid content, followed by A. Senegal, A. polyacantha, and A. seyal gums. A. Senegal gum was rich in serine (0.26%) and aspartic acid (0.22%), while A. seyal gum contained notable amounts of serine (0.22%), aspartic acid (0.14%), and glutamic acid (0.13%). A. polyacantha gum featured aspartic acid (0.24%) and serine (0.21%), and A. laeta gum contained serine (0.43%) and hydroxyproline (0.31%). Overall, the present study underscores the significance of amino acids in gum Arabic composition.

Assessing mineral content in a food commodity is an imperative approach to understanding dietary importance. In this study, the mineral content of gum Arabic, Khorak, and Gond Pak was investigated, focusing on potassium, sodium, calcium, magnesium, and iron. Although minerals constitute only 5% of the typical human diet, they play a crucial role in maintaining normal health and bodily functions. Macrominerals, required in amounts exceeding 100 mg/day, and trace minerals needed in smaller quantities 1–100 mg/day by adults, make up less than 0.01 percent of total body weight [33], were examined. Gum Arabic exhibited the highest levels of potassium (254.22 mg/100g), sodium (118.55 mg/100g), calcium (7976.3 mg/kg), magnesium (2601.5 mg/kg), and iron (76.57 mg/kg) compared to Khorak and Gond Pak. Among the traditional recipes, Khorak had higher potassium (112.49 mg/100g), magnesium (1238.1 mg/kg), and iron (46.55 mg/kg) content than Gond Pak, while Gond Pak contained more sodium (39.53 mg/100g) and calcium (1680.7 mg/kg). Previous studies highlighted the remineralizing effects of gum Arabic, emphasizing its role in enhancing remineralization activities in the body, possibly through polysaccharide salts of potassium, calcium, and magnesium. However, mineral content characterization of gum Arabic is not widespread in scientific literature, potentially due to varietal differences [34, 35].

A substantial number of micronutrients, i.e., vitamins, along with macronutrients, are needed to maintain a sound metabolic system in the human body [36]. The present study focused on assessing the vitamin composition of gum Arabic and its food products. The analysis revealed significant amounts of water-soluble vitamins such as vitamin C and various B complexes (B1, B2, B3, B6, B9, and B12), as well as the presence of fat-soluble vitamin A. Notably, vitamins D, E, B5, and B7 were not detected in any of the samples examined. Interestingly, a prior study by Ajayi *et al.* [37] on the nutritional potential of seeds from the gum Arabic tree found almost all water- and fat-soluble vitamins in the Acacia seeds.

5. CONCLUSIONS

Among the treatments studied, gum Arabic exhibited favorable nutrient content, particularly in minerals, amino acids, and dietary fiber. Khorak demonstrated higher average values for fat, carbohydrates, energy value, certain vitamins (A and C), and amino acids, while Gond Pak stood out for its significant protein and vitamin content. It is noteworthy that some amino acids (cysteine and arginine) and vitamins (pantothenic acid, biotin, D, and E) were not detected in any of the treatments. The study concludes that gum Arabic and its derived

food products, i.e., Khorak and Gond Pak, are highly nutritious, containing essential nutrient components like dietary fiber, minerals, vitamins, and amino acids. It is suggested that gum Arabic, Khorak, and Gond Pak are ideal for human consumption since they are composed of essential nutrients. Therefore, gum Arabic, Khorak, and Gond Pak should be incorporated into the diet, particularly in regions where conditions like malnutrition, kwashiorkor, wasting, stunting, and other diet-specific diseases are frequently prevalent.

6. CONFLICT OF INTEREST

The authors confirm that there are no conflicts of interest.

7. ETHICAL STATEMENT

The present study did not involve any ethical issues concerning human or animal subjects.

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Research Article

Virtual Screening of *Coffea arabica* Phytochemicals as Natural β-Lactamase Inhibitors

Faiz un Nisa and Amina Javid*

Institute of Microbiology and Molecular Genetics, University of the Punjab,
Quaid-e-Azam Campus, Lahore-54590, Pakistan

Abstract: One of the key contributors to antimicrobial resistance is the enzymatic hydrolysis of β-lactam antibiotics by β-lactamases, becoming one of the leading public health challenges. In order to overcome this issue, the current work utilizes advanced *in-silico* grid-based molecular docking and post-docking analysis to identify potential β-lactamase inhibitors from *Coffea arabica* beans. Based on past experimental evidence of coffee's antimicrobial activity, this research aimed to explore the inhibitory potential of its bioactive compounds through computational modeling to identify natural alternatives to synthetic inhibitors. Seventy-three phytochemicals were then screened and molecularly docked by AutoDock against four clinically relevant β-lactamases, namely, AmpC, CTX-M9, CTX-M14, and SHV-1, and subsequently subjected to toxicity and ADMET analysis. Among these, tannin, epicatechin, quercitrin, and quercetin exhibited the highest binding affinities (-8.5 kcal/mol, -7.7 kcal/mol, -8.6 kcal/mol, and -8.6 kcal/mol, respectively), outperforming the reference inhibitor, Avibactam. ADMET analysis also revealed favorable pharmacokinetic, low toxicity, and oral bioavailability of the top-ranked phytocompounds. Collectively, the results indicate the novelty of *C. arabica's* phytochemicals as promising natural β-lactamase inhibitors. However, further *in-vitro* and *in-vivo* studies are required for validating their therapeutic efficacy against resistant bacteria. The current study also establishes a framework for integrating computational approaches in phytochemical research to accelerate antibacterial drug discovery.

Keywords: Antibiotic Resistance, ADMET, β-lactamase Inhibitors, *In-silico* Modeling, Phytochemicals, *Coffea arabica*.

1. INTRODUCTION

Antimicrobial resistance (AMR) has become one of the most severe threats to global public health, resulting in the failure of traditional antibiotic therapies [1, 2]. The production of β-lactamases (βLs) (enzymes capable of hydrolyzing β-lactam antibiotics) represent the most formidable challenge, particularly in Gram-negative pathogens [3]. Moreover, the continuous and rapid evolution of these βLs variants such as SHV-1, TEM-1, AmpC, CTX-M, and NDM-1, and NDM-1, has rendered many clinically relevant β-Lactams ineffective, emphasizing the urgent search for novel inhibitors to restore their efficacy [4]. Natural products are invaluable sources of bioactive compounds of structural complexity and biological specificity with a long history of combating infectious diseases [5]. Over the last few years, phytochemicals of medicinal plants have gained more attention as potential adjuvants or alternatives to traditional synthetic antibiotics [6]. These phytocompounds often have diverse bioactivities (anti-inflammatory, antioxidant, antibacterial, and antiviral), which makes them promising candidates for developing multitarget drug [7]. Plants can produce various secondary metabolites or phytochemicals in response to environmental stress, such as microbial invasion, oxidative stress, high salinity, exposure to ultraviolet radiations, drought or elevated temperatures [8]. Several phytochemicals have shown potential to inhibit βLs, which are bacterial enzymes conferring resistance against β-lactam antibiotics [9]. Among these natural sources, *Coffea arabica* (*C. arabica*) is recognized not only as a globally consumed beverage crop but also as

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* Corresponding Author: Amina Javid <amina-3130305257110@pu.edu.pk>

a reserve of structurally diverse phytochemicals, including alkaloids, flavonoids, saponins, polyphenols, and tannins [10, 11]. *C. arabica* is acknowledged for its abundance of bioactive compounds with potent inhibitory action against various pathogens [12]. Coffee extract contains a variety of bioactive compounds, including chlorogenic acid (CGA), catechins, quercetins, caffeic acids, tannins, and caffeine with inhibitory effects on both Gram-negative and Gram-positive bacteria [13]. Antibacterial components in coffee can inhibit DNA synthesis and inactivate enzymes that are essential to bacterial survival and replication [12]. Despite the increasing evidence of its biological efficacy, the specific molecular mechanisms underlying the antibacterial activities of *C. arabica* remain poorly understood. In particular, the βLs inhibitory potential of its phytochemicals has not yet been thoroughly explored. Computational studies of these natural compounds may, therefore, reveal novel scaffolds for βLs inhibition as well as provide important insights for future antibacterial drug development.

Multi-drug-resistant (MDR) bacteria have become a significant global health challenge due to their widespread resistance to conventional antibiotics [14]. There is an urgent need to discover new antimicrobial drugs to address this issue. A promising recent strategy involves the use of secondary metabolites of plants. Medicinal plants are increasingly recognized as a potential alternative treatment for resistant pathogens compared to synthetic drugs [15]. Plant-derived chemicals possess notable antibacterial properties with fewer adverse effects, making them suitable candidates for antimicrobial drug development. These phytochemicals interact with bacterial systems via several mechanisms such as disrupting the cell membrane integrity, altering the cell permeability, chelating the essential metal ions, inhibiting the nucleic acid or protein synthesis, and directly binding to key bacterial enzymes to block their catalytic activities [16-18]. Thus, natural compounds can be utilized successfully to inhibit bacterial survival and suppress resistance by targeting these mechanisms.

Currently, various advanced bioinformatics tools are available to identify the drug-like properties of phytochemicals more efficiently than traditional, time-consuming experimental procedures [19, 20]. *In-silico* molecular docking is a technique that analyzes the binding interactions between phytochemicals and target enzymes based on binding affinities or docking scores. By combining virtual screening and pharmacoinformatics, the current study aims to investigate the phytochemicals of *C. arabica* for their potential to inhibit βLs, thereby enhancing the efficacy of β-lactam antibiotics against resistant pathogens. The findings are also expected to not only highlight the significant role of *in-silico* modeling in accelerating the drug discovery process but also provide potential scaffolds capable of counteracting βLs-mediated antibiotic resistance.

2. MATERIALS AND METHODS

2.1. Retrieval of Phytocompounds

The Dr. Duke's Phytochemical and Ethnobotanical Database (https://phytochem.nal.usda.gov/) was used to obtain the information about the nature and types of various phytochemicals present in *C. arabica* [21, 22]. A total of 73 phytocompounds belonging to different classes were screened and their 3D structures were downloaded from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in SDF format. These phytochemicals were selected based on their documented abundance, chemical diversity as well as previously reported bioactivities. The structure of Avibactam was also downloaded in SDF format, which was used as reference compound for comparative docking.

2.2. Preparation of Ligands

The SDF format of all the ligands (phytochemicals as well as Avibactam) was then converted into PDB using PyMOL software. Then, all these ligands were saved in PDBQT format with the help of AutoDock Tools v1.5 [21, 23].

2.3. Retrieval and Preparation of Target Enzymes

Four commonly reported βLs, AmpC, SHV-1, CTX-M9 and CTX-M14, were selected as target enzymes in this study based on their high prevalence in increasing β-Lactam resistance in Gram-negative pathogens [24, 25]. The 3D structures of these enzymes were downloaded from Protein DataBank (https://www.rcsb.org/) in PDB format. The PDB

IDs of SHV-1, AmpC, CTX-M9 and CTX-M14 βLs were 4JPM, 5GZW, 3HLW and 6CYU, respectively. The AutoDock tools v1.5.7 were then used to save these structures in PDBQT format, as required by AutoDock Vina [26]. Moreover, the water molecules and native ligands were removed, polar hydrogens were added, and Kollman charges were also assigned to target proteins using AutoDock tools.

2.4. Identification of Active Site

All these enzymes had different ligands already bound to their active sites such as CE4 in CTX-M14, AMP in AmpC, 1OG in SHV-1 and CE3 in CTX-M9 βLs. These ligands provided basic information about the active sites of these enzymes.

2.5. Grid Box Preparation

The grid box was prepared around the already bound ligands using AutoDock tools [27, 28]. Briefly, the grid spacing set at 1Å, the dimensions and central coordinates adjusted at 25 × 27 × 25 Å, x = 8.679, y = 6.851, z = 9.225 for AmpC βL, 27 × 27 × 27 Å, x = 10.101, y = 9.983, z = 10.295 for CTX-M9 βL, 25 × 20 × 23 Å, x = 8.026, y = 12.521, z = 10.42 for SHV-1 βL, and 23 × 21 × 23 Å, x = 15.784, y = 32.761, z = 40.973 for CTX-M14 βL. All this information on grid boxes was then recorded for docking.

2.6. Molecular Docking

Docking was performed via AutoDock Vina using the vina command ("\vina\vina.exe" --config conf. txt --log log.txt) in command prompt [29]. The Lamarckian Genetic Algorithm (LGA) was run at default parameters at 10 distinct sites [19]. To ensure the reliability of docking, native ligands were also redocked into the same binding sites. The output files were saved in PDB format to analyze the binding interactions of ligands at the binding sites of target enzymes, and RMSD values below 2.0 Å were considered acceptable for validation [27].

2.7. Pharmacokinetic Profiles of Phytochemicals

The druglike and toxicity profiles of top scoring phytochemicals were also determined by submitting their canonical SMILES to admetSAR (http://lmmd.ecust.edu.cn/admetsar2/) and PROTOX-II (https://tox-new.charite.de/protox_II/). Compounds were screened according to Lipinski's Rule of Five, Ghose and Veber filters, and toxicity thresholds; molecules satisfying at least four criteria were considered as drug-like candidates [30]. The overall *in-silico* workflow included compound selection, protein preparation, docking validation, screening, and ADMET profiling.

3. RESULTS AND DISCUSSION

The rise in antibiotic resistance is largely attributed to the inappropriate and indiscriminate use of antimicrobial agents. This situation is becoming increasingly critical as many of the bacterial strains have adopted the mechanism of hydrolytic inactivation of β-lactam antibiotic via βLs [3]. This study primarily investigated AmpC, CTX-M-9, CTX-M-14, and SHV-1 βLs, which contribute to the inactivation of β-lactam antibiotics, with the aim of identifying potential inhibitors against these enzymes. Docking analysis of 73 phytocompounds from *C. arabica* against four selected enzymes revealed significant variations in the binding affinities. Among these, tannin (AmpC), quercetin (SHV-1), epicatechin (CTX-M9), and quercitrin (CTX-M14) displayed strongest interactions with binding energies of -8.5, -8.6, -7.7, and -8.6 kcal/mol, respectively, outperforming the reference inhibitor Avibactam. Strikingly, all these phytocompounds showed stable hydrogen and hydrophobic bonding with active side residues such as Lys73, Ser130, Asp123, and Gly32. These amino acid residues are considered essential for the β-lactam hydrolyzing activity of the enzymes.

Plants are well known reservoirs of bioactive compounds guiding modern therapeutic development. Phytocompounds have demonstrated antimicrobial, antiviral, anticancer, anti-Alzheimer, anti-inflammatory, and antioxidant activities [31]. However, screening of these thousands of phytochemicals was traditionally labor-intensive and time-consuming, which, with the advent of various bioinformatic tools, has become comparatively easy [32]. Molecular docking, one of the most widely used bioinformatic tools, screens compounds based on their affinity towards target enzymes. Thus, it enables virtual screening and identification of phytocompounds with the best activity in a cost-effective and time-efficient manner [33]. Molecular docking was also utilized in this

study to identify the phytocompounds of *C. arabica* that exhibit the highest inhibitory activity against target βLs. Table 1 enlists the phytocompounds and their binding affinities for respective target enzymes.

In-silico docking analysis showed that tannin exhibited a strong binding affinity of -7.6 kcal/mol against AmpC βL and was 28.8% more effective compared to synthetic inhibitor Avibactam (-5.9 kcal/mol). Figure 1 shows the 3D interactions of tannin at the binding site of target AmpC βL. Other phytochemicals including quercetin, epicatechin, naringenin, and caffeic acid also showed comparatively high binding affinities for AmpC βL relative to Avibactam (Table 1).

Tannin, a phenolic component naturally occurring in coffee and various types of teas, has been reported to exhibit several beneficial pharmacological properties, including high antioxidant properties and immune system stimulation [34, 35]. Moreover, it may help lower blood cholesterol levels [36]. Several studies have also reported its antibacterial activities against both Gram-positive and Gram-negative pathogens, primarily by disrupting bacterial membranes and inducing cellular damage [37, 38]. Figure 2 presents the 2D interaction of both tannin and Avibactam with active site residues, showing that tannin formed many conventional hydrogen bonds as well as van der Waals interactions with several active site residues, whereas Avibactam formed conventional hydrogen bonds with only four amino acid residues of AmpC βL. The antimicrobial activity of tannin and other polyphenols derived Thai medicinal plant against extended spectrum βL (ESBL) producing *Escherichia coli* has also been reported [39].

Among the tested phytochemicals, quercetin exhibited the highest binding affinity towards SHV-1 βL (-8.6 kcal/mol), followed by tannin (-6.5 kcal/mol), epicatechin (-6.5 kcal/mol), and

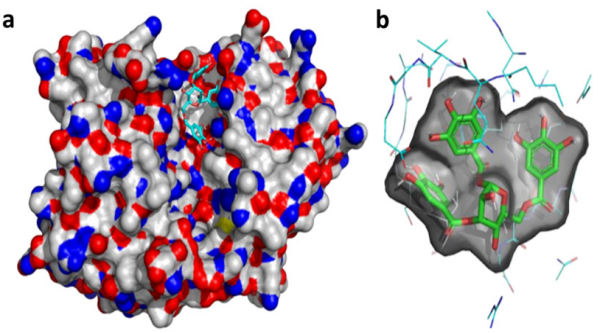

Fig. 1. 3D interactions of tannin at the binding site of AmpC βL.

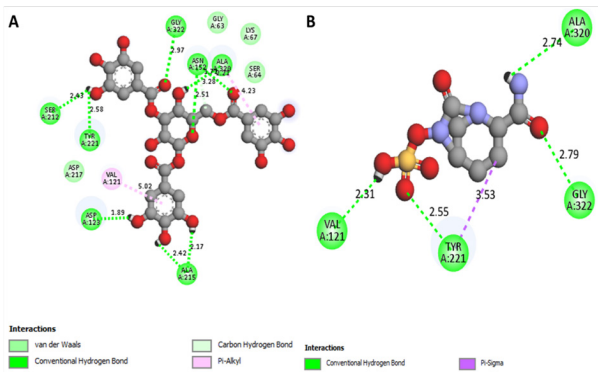

Fig. 2. 2D interactions of tannin (A) and Avibactam (B) with the active site residues of AmpC βL.

Table 1. The binding energies of top scoring phytochemicals as well as synthetic inhibitor (Avibactam).

S. No.	Phytochemicals	Pubchem ID	Binding energies (kcal/mol)			
			AmpC	SHV-1	CTX-M9	CTX-M14
1	Tannin	16129778	-8.5*	-6.5	-7	-6.4
2	Quercetin	5280343	-7.4	-8.6*	-7.2	-7.6
3	Epicatechin	72276	-7.3	-6.5	-7.7*	-7.2
4	Quercitrin	5280459	-7.3	-6.2	-6.5	-8.6*
5	Hyperoside	5281643	-7.3	-6.3	-6.1	-7.7
6	Naringenin	932	-7.1	-6.2	-6.4	-7.1
7	Kaempferol	5280863	-6.9	-6.1	-7	-7.6
8	Caffeic acid	689043	-6.5	-6.3	-7.1	-7.2
9	Avibactam	9835049	-5.9	-5.4	-5.5	-5.2

** highest binding affinities*

quercitrin (-6.2 kcal/mol), as summarized in Table 1. Avibactam, however, exhibited a comparatively lower binding affinity of -5.4 kcal/mol. Figure 3 illustrates the 3D interactions of quercetin within the active site of SHV-1 βL.

Quercetin, also referred to as quercetine or quertine, is a flavanol belonging to the flavonoids subclass, and is found abundantly in various plants such as red onions, broccoli, and various fruits. It's a natural pigment present in significant amounts in several edible plant species. Vafadar *et al.* [40] reported that quercetin shows significant cytotoxic activities against ovarian cancer *in-vitro* and *in-vivo*. Several studies have also reported the therapeutic benefits including anti-inflammatory, antioxidant, anti-diabetic, and anti-microbial effects of quercetin [41]. In addition, quercetin has also exhibited protective effects against COVID-19 due to its immunomodulatory properties [42]. A more recent study by Jian *et al.* [43] has highlighted that quercetin and its derivatives have high potential for treating premature ovary failure (POF), polycystic ovary syndrome (PCOS), endometrial carcinoma (EC) and other gynecological disorders. Figure 4 illustrates the 2D binding interactions of quercetin and Avibactam with the amino acid residues of SHV-1 βL. Lys73 was identified as a common residue interacting with both ligands via van der Waals interaction. The results were in coherence with finding of another study which reported the *in-vitro* inhibition of βL enzyme of *Ficus religiosa* bark extract likely due to presence of quercetin and related flavonoids [9].

Epicatechin, a flavanol abundantly found in tea, cocoa, and several fruits, exhibited the highest binding affinity against CTX-M9 βL, with a docking score of -7.7 kcal/mol. Avibactam, in comparison, exhibited a lower binding affinity of -5.5 kcal/mol for CTX-M9 βL. Although several phytocompounds demonstrated higher binding affinities than Avibactam, only the top-performing compounds are listed in Table 1. Figure 5 illustrates the 3D docking interactions between CTX-M9 βL and epicatechin.

Epicatechin is a monomeric flavonoid with several reported therapeutic benefits. Seo *et al.* [44] reported that epicatechin and gallic acid can enhance muscle mass and potentially ameliorate age-related muscle decline. Epicatechin and its derivatives also exhibit potent antioxidant and anti-inflammatory activities, significantly improving neuronal health following brain injury [45, 46]. 2D interactions of epicatechin and Avibactam within the active site of the CTX-M9 βL are represented in Figure 6. Epicatechin interacted with several active site residues of CTX-M9 βL via conventional and non-conventional hydrogen bonds, van der Waals forces, and hydrophobic interactions. Avibactam, on the other hand, did not establish any conventional hydrogen bonding. Asn132 and Thr216 were identified as common residues interacting with both epicatechin and Avibactam, though the nature of interactions differed. Buchmann *et al.* [47] also highlighted the synergistic potential of using epicatechin-antibiotic combination in fighting against βL-producing ESKAPE pathogens through time-kill assay. Additionally, epicatechin and its derivatives also show significant inhibitory activities against *Staphylococcus aureus* by suppressing its biofilm formation and βL enzymatic activity [48].

Quercitrin exhibited the highest binding affinity of -8.6 kcal/mol for CTX-M14 βL. In contrast, Avibactam displayed a comparatively

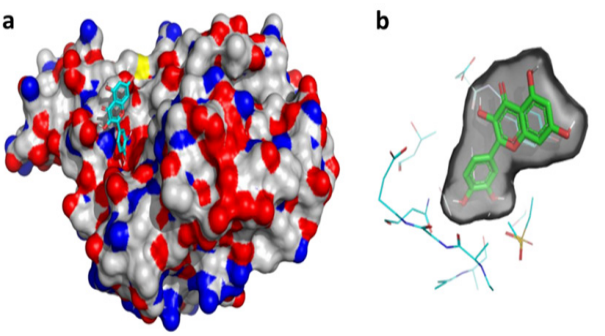

Fig. 3. 3D interactions of quercetin at the binding site of SHV-1 βL.

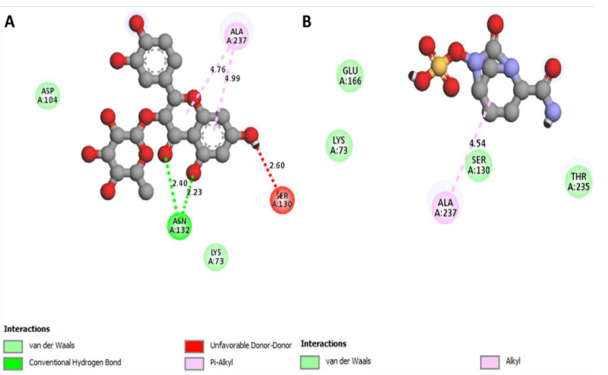

Fig. 4. 2D interactions of quercetin (A) and Avibactam (B) with the active site residues of SHV-1 βL.

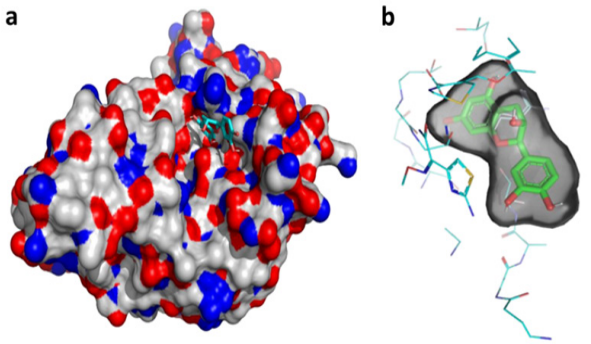

Fig. 5. 3D interactions of epicatechin at the binding site of CTX-M9 βL.

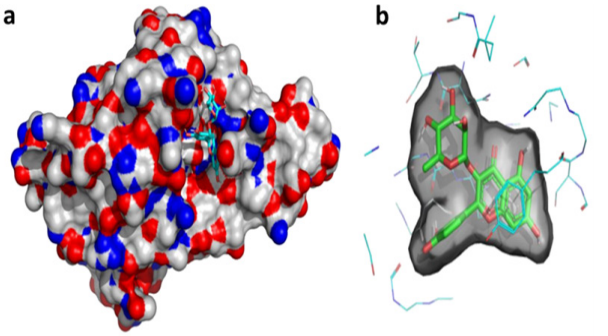

Fig. 7. 3D interactions of quercitrin at the binding site of CTX-M14 βL.

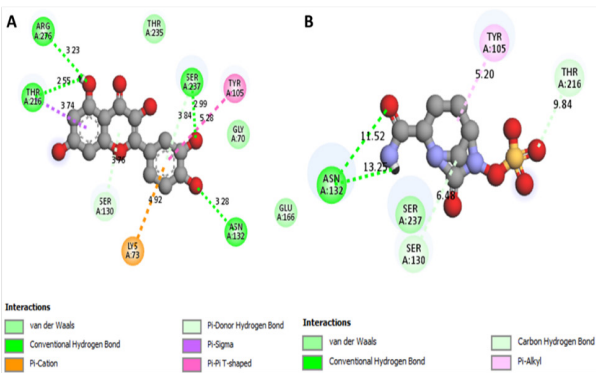

Fig. 6. 2D interactions of epicatechin (A) and Avibactam (B) with the active site residues of CTX-M9 βL.

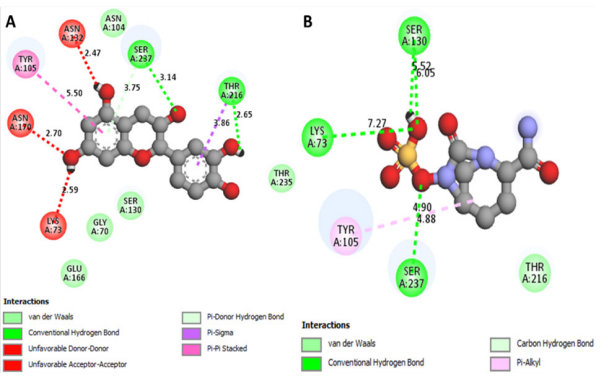

Fig. 8. 2D interactions of quercitrin (A) and Avibactam (B) with the active site residues of CTX-M14 βL.

lower binding energy of -5.2 kcal/mol. Several other *C. arabica* phytocompounds also demonstrated stronger affinities than Avibactam, as summarized in Table 1. The 3D interactions of CTX-M14 βL and quercitrin are shown in Figure 7, illustrating hydrogen bonds and hydrophobic contacts with key catalytic site residues.

Quercitrin is also a flavonoid glycoside derivative of quercetin linked with rhamnose sugar. Like other phytocompounds, it is ubiquitously found in several grains, leaves and other parts of vegetables and fruits [49]. Quercitrin has been reported to have significant anti-inflammatory and anti-tumorigenic activities, especially against prostate and bladder cancers [50, 51]. Li *et al.* [52] reported that quercitrin induced treatments significantly reduced tumor cell viability in lung adenoma induced mice. It is also reported to show hair stimulating activities by activating the expression of growth factors through MAPK pathway in follicle cells [53]. The 2D interactions of quercitrin and Avibactam at the binding site of CTX-M14 βL are shown in Figure 8. Lys73, Tyr105, Ser130, Thr216, and Ser237 were some of the common amino acids of CTX-M14 βL interacting with both quercitrin and Avibactam in different types of bonding interactions.

The pharmacological profiles of top-scoring phytochemicals and Avibactam were also evaluated to analyze their drug-likeness and therapeutic potential [30]. This analysis was based on Lipinski's Rule of Five, which evaluates key physicochemical properties such as molecular weight, hydrogen bond donor and acceptor counts, molar refractivity, and other relevant descriptors to predict the potential of a compound to serve as a drug candidate [54-56]. As summarized in Table 2, majority of the phytochemicals satisfied the drug-likeness criteria. The favorable pharmacokinetic properties of these phytocompounds also highlight their potential as lead compounds for further therapeutics development. In summary, *C. arabica* harbors diverse array of phytochemicals with significant potential to inhibit βLs responsible for antibiotic resistance in Gram-negative pathogens. These compounds may not only enhance the activity of β-lactam antibiotics but also help restore their efficacy against resistant bacterial strains.

Table 2. Drug-like properties of top scoring phytocompounds and Avibactam.

Compounds	Lipinski's rules					Rule of 5's violations
	MW	Log $P_{o/w}$	Molar Ref.	HBA	HBD	
	< 500	≤ 5	40-130	≤ 10	≤ 5	
Tannin	1701.21	4.84	391.5	46	25	4
Quercetin	302.24	1.99	78.04	7	5	0
Epicatechin	290.27	1.55	74.33	6	5	0
Quercitrin	448.38	0.49	109	10	7	1
Hyperoside	464.38	-0.54	110.16	11	8	2
Naringenin	272.26	2.51	71.57	5	3	0
Kaempferol	286.24	2.28	76.01	6	4	0
Caffeic acid	180.16	1.2	47.16	4	3	0
Avibactam	265.25	-1.53	60.27	5	2	0

4. CONCLUSIONS

In conclusion, plant-derived natural compounds are receiving growing attention in the field of drug discovery due to their diverse therapeutic benefits. The integration of bioinformatic approaches in this field has also significantly accelerated the process of identifying and characterizing potential drug candidates from plants. This study also employed multiple *in-silico* approaches to screen and identify the phytocompounds of *C. arabica* that can inhibit the βLs, which contribute significantly to antibiotic resistance in Gram-negative pathogens such as *E. coli* and *Klebsiella pneumoniae*. Among the screened phytocompounds, tannin (-8.5 kcal/mol), epicatechin (-7.7 kcal/mol), quercetin (-8.6 kcal/mol) and quercitrin (-8.6 kcal/mol) displayed stronger binding affinities for the four target enzymes (AmpC, CTX-M9, SHV-1 and CTX-M14 βLs), respectively, as compared to the Avibactam, a synthetic βLs inhibitor. Moreover, the pharmacological analysis of the top scoring phytochemicals also confirmed their drug-like properties and safety profiles. Further *in-vitro*, *in-vivo*, and molecular dynamics studies are required that can validate the potential therapeutic benefits of these natural compounds in controlling β-Lactam resistance.

5. CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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9. UNESCO. Global Education Monitoring Report 2024/5: Leadership in education–Lead for learning. *United Nations Educational, Scientific and Cultural Organization, Paris, France* (2024). https://digitallibrary.un.org/record/4066661?ln=en&v=pdf

10. L.M. Highland and P. Bobrowsky. The landslide handbook–A guide to understanding landslides. Circular 1325. *US Geological Survey, Reston, Virginia* (2008).

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11. M. Khalid, A.B. Majid, F. Mansour, and C.R. Smith. Word Representations with Recursive Neural Networks for Morphology. *27^{th} European Conference on Signal Processing, (2^{nd} - 6^{th} September 2021), Madrid, Spain* (2021).

g. A Degree Thesis

12. M. Afzal. Investigation of structural and magnetic properties of nanometallic Fe-Mn Alloys. Ph.D. Thesis. *Quaid-i-Azam University, Islamabad, Pakistan* (2023).

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