

Quantitative analysis of Insecticide in leafy vegetables grown along the railway track in Mumbai and MMR region

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Abstract:

Agriculture is a key component of the Indian economy. Yet, due to developmental activities, there has been encroachment over agricultural land. Owing to the shortage of land in Mumbai City and the adjoining MMR, agricultural practices are carried out in the space adjacent to the railway tracks. Cultivation of leafy vegetables such as Spinach and Amaranth is gaining ground due to increasing demand and hence the cultivation is carried out throughout the year. The farmers use chemical fertilizers and pesticides to maximize yield and increase profitability. Leafy vegetables are a staple food and a significant part of a person's diet and are rich in vitamins, fiber, and minerals, and low in calories. Excessive use of chemical fertilizers and pesticides leads to pollution of water and soil which can be toxic to consumers. The present study was carried out to quantify the Imidacloprid insecticide residue in leafy vegetables grown near railway tracks in Mumbai City (Central and Harbour Lines). For this purpose, HPTLC method was used due to its ability to provide precision, quantification, and accuracy in results. In Monsoon, late post-monsoon, and Pre-Monsoon, the maximum residue limit of Imidacloprid was found to be 3.973 mg/kg in Amaranth at the Central Line; 2.199 mg/kg at the Harbour Line and 1.919 mg/kg at the Central Line, respectively. Results indicate that there is a higher concentration of insecticide in grown vegetables. Consumption of such contaminated vegetables daily poses a threat to consumers' health.

Keywords: Imidacloprid, insecticides, HPTLC, Railway track, etc.

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1.0 INTRODUCTION

India's agricultural sector, playing a crucial role in the national economy as the second-largest vegetable producer globally (IBEF, 2022), faces unique challenges in urban areas like Mumbai and the adjacent Mumbai Metropolitan Region (MMR). Factors such as land scarcity, unemployment, and developmental projects have led to the emergence of non-traditional farming practices in these regions. Notably, farmers have resorted to cultivating leafy vegetables, including spinach, amaranth, radish, fenugreek, red amaranth, taro, etc. along the railway tracks (Vazhacharickal P.J. *et.al.*, 2013). These leafy vegetables are economically viable crops with high yields and low production costs (S. Khan *et.al.*, 2013). These have a significant place in the regular diet of individuals as a staple food. They are rich in essential nutrients, including vitamins, iron, and dietary fibers, providing numerous health benefits (Zang Q. *et. al.*, 2021). Their consumption can be associated with the reduction of cholesterol levels, prevention of cardiac problems, and the management of high blood pressure. (Elske M. Brouwer-Brolsma, *et. al.*, 2020)

The cultivation of leafy vegetables near railway tracks poses unique challenges and risks for farmers. These challenges are due to the presence of drainage outlets, industrial outlets, construction sites, slums, and dumping areas near railway lines and agricultural land (Jadhav & Kurve, 2023). These unsanitary surroundings are responsible for pest infestations on vegetables, resulting in decreased crop yield and compromised produce quality. Consequently, farmers tend to rely heavily on the excessive application of chemical fertilizers and pesticides to counteract these challenges and maintain higher productivity.

Pesticides like neonicotinoids, organophosphates, cypermethrin, pyrethroid, etc. are used in combination to protect the crops in these areas. Imidacloprid is one of the commonly used insecticides in the study areas for vegetable protection. Imidacloprid insecticide belongs to the class of chloronicotinyl neonicotinoid insecticides and is widely used in agricultural practices for vegetable protection. Imidacloprid targets the nervous systems of insects by binding to nicotinicacetylcholine receptors, disrupting nerve cell transmission (NPIC: National Pesticide Information Centre, 2010). Although Imidacloprid is relatively low in toxicity to mammals and is noncarcinogenic, it does pose risks to non-target organisms in the fields. Of particular concern is the fact that nicotinic-acetylcholine receptors, which Imidacloprid targets in insects, are also present in humans. Excessive use of agrochemicals like Imidacloprid without due precautions raises concerns about health risks for humans, non-target organisms, and the environment. Studies have shown that Imidacloprid exposure is linked to neurotoxic effects, developmental delays, endocrine disruption, loss of coordination, tremors, decreased activity, reduced body temperature, and coma or death at high doses and organ damage in humans particularly for women of childbearing age, infants, and children. (Lohstroh and Koshlukova, 2021; Eiben, 1991; Eiben and Kaliner, 1991; Maele-Fabry G, et.al., 2017; Oulhote Y., et.al., 2013; Bonmatin JM, et.al., 2015).

The consumption of pesticide-contaminated leafy vegetables on a regular basis poses threats to human health and the environment. Furthermore, the proximity of railway tracks to urban areas raises concerns about the potential contamination of vegetables grown here from pollutants such as heavy metals, exhaust emissions, and industrial waste. This necessitates rigorous monitoring and evaluation of the quality of commodities cultivated in these regions, considering the encroachment of agricultural operations along the railway tracks. Therefore, this study focuses on assessing the levels of contamination of Imidacloprid insecticide residues in soil, water, and vegetables grown along the railway tracks of Mumbai city. The standard analytical technique of HPTLC was employed to determine the levels of Imidacloprid residue. The study also examines the seasonal variation of these levels at all the study locations, including Central, Harbour, Trans-harbour, and Organic Farming areas. Additionally, the study compares unconventional farming practices with Organic Farming practices in Mumbai. Furthermore, the study investigates the detrimental impacts of Imidacloprid insecticides on humans and the environment, so as to provide valuable recommendations for policymakers, farmers, and stakeholders involved in promoting safe and sustainable farming practices.

2. 0 RESEARCH METHODOLOGY

2.1 STUDY AREA

Mumbai railway lines, which connect the Navi Mumbai and MMR districts, are one of Asia's oldest railway networks. More than 120 stations are operational on this railway network (S. Nambiar, 2016). The Mumbai railway system is divided into two lines: the Western Line and the Central Line. The Central Line in Mumbai operates three major lines: the harbour, transharbour, and Nerul Uran lines. The MMR region covers an area of more than 4355 km² in Mumbai, Maharashtra, India. (P. Vazhacharickal *et. al.*, 2013). In this study, vegetables, soils, and water samples were collected from the agricultural lands near Mumbai's Central Line, Harbour Line, and Trans Harbour Line and compared to Organic Farming practices existing in Mumbai.

Geographical coordinates for study locations are given below in table no.1

Sr.	Railway Lines	Codes	Study Locations	Latitude & Longitude
No.				
1.	Central Line	CL	Kalwa	19.1968 N, 72.9977 E
2.	Harbour Line	HL	Kharghar	19.0265 N, 73.0595 E
3.	Trans- Harbour Line	THL	Airoli	19.1585 N, 72.9994 E
4.	Organic Farming	OF	Kalwa	19.2100 N, 73.1849 E

Table No. 1 Geographical coordinates of study locations and codes

The study period was divided into four seasons such as Late Post Monsoon (LPM), Pre-Monsoon (PM), Monsoon (M), and Early Post Monsoon (EPM). Random sampling techniques were used to collect vegetable samples of Spinach and Amaranth from all study locations. Soil samples were collected from the study locations using random sampling, while water samples were collected from all study locations using the grab sampling technique. (A. Facchi *et.al.*, 2007; Almaleeh A., 2022). The selected study locations are in close proximity to various sources of potential contamination, such as construction sites, industrial outlets, drainage pipelines in residential areas, slums, dumping areas, and marshes. The presence of such unhygienic environments increases the susceptibility of vegetables to pest infestations. Consequently, to mitigate losses and enhance profitability, excessive use of chemical pesticides and fertilizers is commonly practiced in these areas.

2.2 REAGENTS AND STANDARD

Chromatography-grade solvents, including water and methanol, were used for sample extraction, while n-hexane and acetone (Merck (Mumbai, India) served as the mobile phase. Imidacloprid (Sigma Aldrich (Mumbai, India), in the form of analytical reagent-grade chemicals, was exclusively utilized in the experiments.

2.3 VEGETABLE SAMPLES COLLECTION AND EXTRACTION METHOD:

Vegetable samples, including Spinach (*Spinacia oleracea*) and Amaranth (*Amaranthus spp.*), were collected from the study locations. The extraction method involved finely chopping 1 kg of Spinach and Amaranth samples, immersing them in a solvent mixture of Methanol and Water (80:20 v: v), and allowing them to soak for 24 hours with regular stirring. Excess solvent was evaporated in a water bath at 50-100 °C, and the resulting extracts were stored in airtight plastic bottles. After subjecting the vegetable extracts to sonication bath treatment at 60 °C for 20 minutes and centrifugation at 3000 rpm for 5 minutes, they were ready for High-Performance Thin-Layer Chromatography (HPTLC) analysis to identify and quantify pesticide residues in the samples (B. Narshkumar et.al., 2018; A. Bhargav et.al., 2021).

(*Note:* It is imperative to refrain from washing the vegetables with water before the extraction process to avoid potential alterations or dilution of the targeted pesticide residues)

2.4 SOIL SAMPLE COLLECTION AND EXTRACTION METHOD:

Soil samples were collected from study locations using random sampling at a depth of 0-30 cm. After air-drying for 2 days to remove moisture, extraneous materials were carefully removed. The dried soil samples were pulverized and sieved to obtain a fine powder. For Imidacloprid estimation, 0.5 grams of soil were transferred to microcentrifuge tubes, and 1 ml of methanol was added. Ultrasonic sonication was applied for 20 minutes, followed by centrifugation at 3000 rpm for 5 minutes. The resulting supernatant was collected in vials for HPTLC analysis.

2.4 WATER SAMPLE COLLECTION AND EXTRACTION METHOD:

Water samples were collected using plastic bottles with proper labeling. To prevent contamination, the bottles were rinsed twice with the respective water samples before collection. After labeling, the water samples were stored in a laboratory refrigerator at 4°C. For Imidacloprid residue quantification, 1 ml of each water sample was transferred to centrifugal tubes and centrifuged at 3000 rpm for 5 minutes. The resulting samples were then collected in vials for subsequent HPTLC analysis.

2.5 HPTLC FINGERPRINTING ANALYSIS

High-Performance Thin-Layer Chromatography (HPTLC) was utilized as a densitometric method to quantify the Imidacloprid pesticide in methanolic extracts of Soil, Spinach, Amaranth, and Water samples obtained from the study locations (CL, THL, HL, and OF) across four seasons (LPM, PM, M, and EPM). The HPTLC analysis was performed using a CAMAG (Muttenz, Switzerland) HPTLC system equipped with a LINOMAT 5 applicator fitted with a 100 µl syringe, CAMAG TLC scanner, and WIN CATS (DESKTOP-JKQIKTT, version 3.1.21109.3) software.

The stationary phase used in this method was a Merck Silica gel 60 F254 (20×10 cm) pre-coated aluminum sheet. A suitable volume of the methanolic extract solution from the vegetables, soil, and water samples was spotted 8 mm from the bottom and 8 mm from the side edges of the plate. After the application, effective separation was achieved using the mobile phase, n-Hexane: Acetone (13:7 v/v). The developed plate was then scanned using a TLC plate scanner at a wavelength of 276 nm for densitometric analysis.

The optimized chamber saturation time for the mobile phase was set at 20 minutes at room temperature. The chromatographic run length was 70 mm from the bottom edge of the plate. Following the development, the HPTLC plates were dried in an oven at 60°C for 5 minutes to ensure the complete removal of the mobile phase. Densitometric scanning was performed using a TLC scanner equipped with WIN CATS (DESKTOP-JKQIKTT, version 3.1.21109.3) software in reflectance absorbance mode. The scanning parameters included slit dimensions of 6 mm \times 0.45 mm, a scanning speed of 20 mm/s, a data resolution of 100 µm/step, an optical filter (second order), and a filter factor (Savitzky-Golay 7). The plates were scanned at 254 nm and selected experimentally based on the distinctive absorption spectra of the compounds between 200 and 400 nm. Furthermore, the plate was placed in a photo-documentation chamber (CAMAG REPROSTAR 3) and images were captured under visible light, UV 366 nm, and UV 254 nm. Peak numbers, their height and area, peak display, and peak densitogram were recorded and analyzed using the software.

2.6 STATISTICAL ANALYSIS

The statistical analysis was used to assess the seasonal variation of Imidacloprid pesticide content in soil, water, and leafy vegetable samples collected from CL, THL, HL, and OF study locations. ANOVA test was used to examine the seasonal variations within the results if any.

3. RESULTS AND DISCUSSION



Graph No. 1 Imida residues in Soil



Graph No.3 Imida residues in Amaranth



Graph No. 2 Imida residues in Water



Graph No.4 Imida residues in Spinach

The presence of excessive pesticide residue levels in soil, water, and leafy vegetables grown near railway tracks in Mumbai has been shown to have negative impacts on human health and the environment, as noted by Khan *et al.* (2013). Consumption of such contaminated leafy vegetables on a regular basis for the long term may pose a threat to the health of the consumers. Therefore, the present study was conducted to quantify the maximum residue limit (MRL) of Imidacloprid insecticide in soil, water, and leafy vegetables using the HPTLC technique. HPTLC technique is commonly used for the detection and quantification of pesticide residues in vegetable samples due to its convenience and cost-effectiveness. Previous research conducted by Pujeri *et. al.* (2016) and Khan M. S. *et.al.*, (2011) also utilized the HPTLC technique to detect and quantify pesticide residues in fruits and vegetable samples, highlighting its effectiveness.

During the study period, the concentration of Imidacloprid residues in the soil across all study locations ranged from 0 to 1.777 ppm. Maximum residue levels for Imidacloprid in the soil was observed in the agricultural area at the Central Line during the LPM season. (Graph no. 1) The higher concentration of Imidacloprid in soil during the LPM season. can be attributed to its slow degradation property in the soil. This slow degradation of Imidacloprid in the soil is caused by factors such as low microbial activity, reduced soil temperature, and limited organic matter leading to the persistence and accumulation of Imidacloprid residues in the soil (Goswami et. al., 2016; Sarkar M. A. et. al, 2001). Imidacloprid residue was found to be lower in the soil during the PM, Monsoon, and EPM seasons due to several factors. During the PM season, the lower residue levels of Imidacloprid in the soil can be attributed to reduced pesticide usage due to reduced pest pressure. Consequently, the reduced application of Imidacloprid results in lower residue concentrations (Goswami et al., 2016). The Monsoon season, characterized by heavy rainfall, can lead to the leaching and dilution of pesticide residues in the soil, resulting in lower levels of Imidacloprid (Saha et.al., 2016; Gutpa S. et. al, 2002). Additionally, increased microbial activity during the Monsoon can contribute to the degradation of Imidacloprid residues (Saha et.al., 2016). The EPM season may also witness decreased pesticide usage, contributing to lower Imidacloprid residue levels (Goswami et.al., 2016).

The concentration of Imidacloprid residues in water varied from 0 to 1.96 ppm across all study locations (Graph no.2). HL recorded the highest residue level of 1.96 ppm during the EPM season, followed by the CL with 1.259 ppm during the Monsoon season. These elevated residue levels can

be attributed to the increased use of pesticides in agricultural activities during these seasons, driven by higher pest pressure (Yo *et al.*, 2016). Further heavy rainfall during the Monsoon season contributes to greater agricultural runoff and the leaching of Imidacloprid pesticides from fields into water sources (Singh *et al.*, 2017; Liu Z. *et. al.*, 2022). As a result, higher residue levels of Imidacloprid were observed in water bodies during the EPM and Monsoon periods. The abundant rainfall also leads to increased water volumes in rivers and other water bodies, potentially diluting the concentration of Imidacloprid residues (Lürling & Scheffer, 2007) yet higher levels were observed during monsoon suggesting over application of pesticides containing Imidacloprid. During the LPM and PM seasons, there is a decrease in pesticide application and agricultural runoff resulting in negligible residue levels of Imidacloprid (Zhang & Wania, 2014).

Plants are known to absorb pesticide residues from the surrounding environment. During the study period, Imidacloprid residue in Amaranth was found to be in the range of 0-3.973 ppm during the present study. (Graph No. 3). The maximum residue level of Imidacloprid was recorded during the Monsoon season which was 3.973 ppm. The maximum residue level of Imidacloprid insecticide i.e., 3.973 ppm), was observed at a central line during the Monsoon season which is beyond the maximum residue limit of Amaranth set by the US EPA i.e., 3.5 ppm. The concentration of Imidacloprid residue in Spinach ranged from 0 to 3.395 ppm, with the highest level observed during the Monsoon season at the CL (Graph no. 4). The higher concentrations of Imidacloprid insecticide in both the vegetables is of the intensified use of pesticide by the farmers during Monsoon season as it is characterized by high pest pressure as conditions are favorable for pest outbreaks. The heavy rainfall during the Monsoon season can facilitate the absorption of Imidacloprid \by plants through their leaves (foliar uptake) (Chauhan, R., & Singh, R., 2018; Chauhan R. et. al., 2021). Rainfall can wash off pesticide residues from plant surfaces and allow them to enter the soil, which may then be taken up by the plants. The increased moisture and humidity during the Monsoon season can enhance the absorption and translocation of Imidacloprid within the plants, resulting in higher residue levels in Amaranth and spinach. (Singh, B., Singh, R. S., & Jangid, R., 2017: Sondhia, S., & Singh, B., 2011). This can contribute to higher concentration levels in the plants during this period.

The single-factor analysis of variance (ANOVA) confirmed the statistically significant seasonal variations in Imidacloprid residues in soil across all study locations (F = 43.989, p < 0.05)

throughout the study period. Whereas no statistically significant seasonal variation was observed in the Imidacloprid content in water (F = 3.197, p = 0.062). and Amaranth (F = 3.199, p = 0.0622) and Spinach (F = 0.6384, p = 0.6045).



Graph no.5: Seasonal variation in residue concentration of Soil, Water, Amaranth, and Spinach.

The residual concentration of Imidacloprid within the test vegetables was seen throughout the study period while the Monsoon concentrations were higher than the Maximum Residue Limit (MRL) of 3.5 ppm prescribed by the US EPA during the monsoon season. The residue concentration in soil was higher during the LPM whereas for water it was high during the EPM. The minimal variation in the concentrations of Imidacloprid residues in soil, water and both the test vegetables (Amaranth and Spinach) can be attributed to factors such as rainfall, agricultural runoff, irrigation practices, and use of insecticides. A trend is observed where the Imidacloprid concentration in water and the vegetables is seen to increase after the PM season and a dip in concentration in vegetables is seen after Monsoon whereas the concentration in soil remains low after LPM. This trend could be due to the uptake of Imidacloprid by the plants and the seepage of pesticides through rainwater in the nearby water bodies.

4. CONCLUSION

The cultivation of leafy vegetables near railway tracks in the Mumbai region poses significant concerns regarding quality and contamination risks. The presence of Imidacloprid pesticide residues in soil, water, and leafy vegetables across all study locations throughout the study period, including Organic Farming, underscores the critical need to implement sustainable practices in these regions to protect food safety, consumer health, and the environment. (Chang et. al., 2018; Cheng et. al., 2022). The excessive use of agrochemicals, particularly during the Monsoon season, further exacerbates the issue, leading to contamination of nearby water bodies. This not only affects the yield and quality of vegetables but also poses hazards to the environment and biodiversity of wetlands and rivers (Starner, et.al, 2012). To address these challenges, regular monitoring of water, soil, and food produced in these areas should be implemented as a mandatory practice for railway track farming (Doshi & Zele, 2014). Additionally, converting these farming lands into railway gardens with floriculture or ornamental plants can provide a safer alternative for citizens (The Indian Express PTI, 2022). It is imperative to adopt sustainable practices, avoid the uncontrolled use of chemical fertilizers and pesticides, and prioritize the preservation of soil and water quality. By implementing these measures, we can ensure the production of safer and healthier agricultural products while safeguarding the environment and promoting sustainable farming practices.

5. RECOMMENDATIONS

This study proposes a set of scientific recommendations for achieving sustainable railway track cultivation in Mumbai and the surrounding region. Specifically, the focus is on utilizing sewage water for the cultivation of leafy greens intended for local markets. The concern addressed is the potential adverse impacts of excessive fertilizer and pesticide use on consumer health and the environment. The recommendations encompass several key aspects, including farmer education on sustainable agricultural practices, the establishment of regulatory guidelines to govern sewage water usage with prescribed limits for chemical inputs, the implementation of robust monitoring systems to assess water quality and detect crop residue contamination, conducting research to explore Organic Farming techniques and identify suitable crop varieties, engaging stakeholders to raise awareness and garner support for sustainable practices, fostering collaborations among farmers and institutions, providing economic incentives to encourage the adoption of sustainable

practices, and promoting continuous improvement through the incorporation of ongoing research findings and stakeholder feedback. Implementing these scientific recommendations will contribute to the achievement of safe and efficient railway track cultivation, ensuring sustainability, consumer safety, and environmental protection.

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