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Azadirachtin as a sustainable tool for zero pesticide residue production: Residue dissipation in open-field tomato production

Tahseen Chikte[1]*, *Václav Psota*[3], *Michal Kumšta*[2], *Tomáš Kopta*[1]

[1]*Faculty of Horticulture, Mendel University, Lednice, Czech Republic*
[2]*Department of Viticulture and Enology, Faculty of Horticulture, Mendel University, Lednice, Czech Republic*
[3]*Farma Bezdínek s.r.o., Dolní Lutyně, Czech Republic*

**Corresponding author: xchikte@node.mendelu.cz*

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Abstract: The growing demand for vegetables free from pesticide residues has fuelled the search for sustainable pest management solutions. This study assessed the efficacy of azadirachtin, a neem-derived biopesticide, in achieving no detectable pesticide residues in tomato production under open-field conditions. The experiment, conducted from April to September 2024, included a systematic application and residue analysis using liquid chromatography-mass spectrometry (LC-MS). The results showed that azadirachtin degraded rapidly, with residual levels in leaves, green fruits, and mature fruits falling below the detection threshold (0.01 mg/kg) after 8–10 days following treatment. The statistical analysis revealed strong time-dependent residue dissipation, with little systemic buildup in fruit tissues. The findings suggest that azadirachtin is a viable, environmentally friendly alternative to synthetic pesticides, aligning with food safety requirements and customer preferences for pesticide residue-free fruit. Future research should investigate the ecological factors that affect degradation rates to optimise its application in diverse agro-climatic conditions.

Keywords: pesticide-free vegetables; sustainable pest management; neem-derived biopesticide; liquid chromatography-mass spectrometry (LC-MS); residue analysis; synthetic pesticides; degradation rate

The production of tomatoes is essential to world agriculture, as they are one of the most widely consumed vegetables and play a significant role in both industrial food production and home diets. Tomato production and consumption are exceptionally high in Europe, where countries such as Italy, Spain, and the Netherlands lead the world in both open-field and greenhouse production (Eurostat 2025). Tomato crops are susceptible to various pests, including arthropods, pathogens, nematodes, and weeds, which can drastically affect yield and quality. According to the statistics, these pests have the potential to reduce feasible tomato yields by 77.7% if appropriate crop protection measures are not adopted (Zalom 2003). For example, the tomato leafminer (*Tuta absoluta*) has become a glob-

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al issue, damaging 60% of tomato crops globally, an alarming increase from just 3% a decade earlier, partly due to incorrect pesticide use (Giménez 2020). Uncontrolled insect infestations jeopardise food security and directly influence food availability and quality. However, excessive pesticide usage raises worries about crop residue accumulation, posing health hazards to consumers and threatening environmental sustainability. Prolonged exposure to these residues has been linked to, for example, endocrine disruption and other toxicological effects, which have serious consequences for consumer health (Vandenberg et al. 2020). A 2024 investigation by the Pesticide Action Network (PAN) revealed that 48 pesticides, including 29 related to endocrine disruption, were not permitted for use in domestic foods imported into the UK (Horton 2024).

Moreover, previous studies have demonstrated that even low-level exposure to certain pesticides can impact the human hormone system, leading to immunological disorders, developmental abnormalities, and reproductive issues (Mnif et al. 2011; Uwamahoro et al. 2024). To address these problems, farmers and policymakers have prioritised attaining zero pesticide residue standards in vegetable crop production as awareness of environmental sustainability and food safety increases (EFSA et al. 2024). Such standards are voluntary and are mostly defined as zero, or 0.01 mg/kg of pesticide residue. In the European Union, such a definition is legislatively defined [(EC) Regulation 396/2005]. This approach meets the stringent safety standards of regulatory bodies and aligns with the increasing consumer demand for more sustainable and health-conscious agricultural practices (Hou & Wu 2010). Azadirachtin is a promising alternative to synthetic pesticides, a bioactive compound derived from the neem tree (*Azadirachta indica*), which has been widely recognised for its potent insecticidal properties and ecological safety citation.

Azadirachtin is primarily an insect growth regulator that disrupts moulting, inhibits feeding, and reduces fertility in a wide range of agricultural pests, including aphids, thrips, and caterpillars (Bernardes et al. 2017; Fernandes et al. 2019; Lin et al. 2021). Azadirachtin promotes ecological balance while protecting crops due to its low toxicity to non-target species and compatibility with integrated pest management (IPM) systems (Savi et al. 2024). Azadirachtin is biodegradable, resulting in fewer pesticide residues and reduced treatment frequency (Ferdenache et al. 2019; Kilani-Morakchi et al. 2021). Its systemic protection and combination with biological control approaches strengthen its significance in organic farming by promoting the conservation of beneficial species (Bernardi et al. 2013; Biondi et al. 2013). Moreover, its rapid degradation minimises the risk of non-target toxicity, a primary environmental concern associated with synthetic pesticides. Many conventional chemical pesticides have been shown to have a negative impact on onollinators, microbial communities, and aquatic ecosystems by persisting in soil and water over time (Goulson 2013). Azadirachtin, a botanical pesticide approved for use in organic agriculture, offers a safer and more sustainable pest management solution compared to conventional insecticides due to its rapid disintegration and short residual action, thereby reducing the risk of unwanted ecological impacts (Isman 2006). Its quick dissipation is crucial for zero-residue vegetable production methods, where reducing pesticide residues is critical. Therefore, this study aims to determine the timeframe required to achieve zero detectable pesticide residues using azadirachtin as a biopesticide under open-field conditions. It is focused on the hypothesis that azadirachtin is not systematically distributed to tomato fruits through the green parts, primarily the leaves.

MATERIAL AND METHODS

Site selection

The experiment was conducted in an open field at the Faculty of Horticulture in Lednice from April to September 2024. During the experiment, the mean monthly temperature, humidity, sunshine, and precipitation were recorded using automatic sensors near the experimental field (Figure 1).

Plant material, site conditions, and pesticide application

The experiment was conducted using tomato cultivar Valdo (SEMO), which is very early, with date-like red fruits weighing 20–25 g. The seedlings were grown in a greenhouse with daytime temperatures ranging from 21 °C to 29 °C and nighttime temperatures between 15 °C and 20 °C. Furthermore, the healthy seedlings were transplanted on May 20, 2024 in an open field with 60 cm spacing be-

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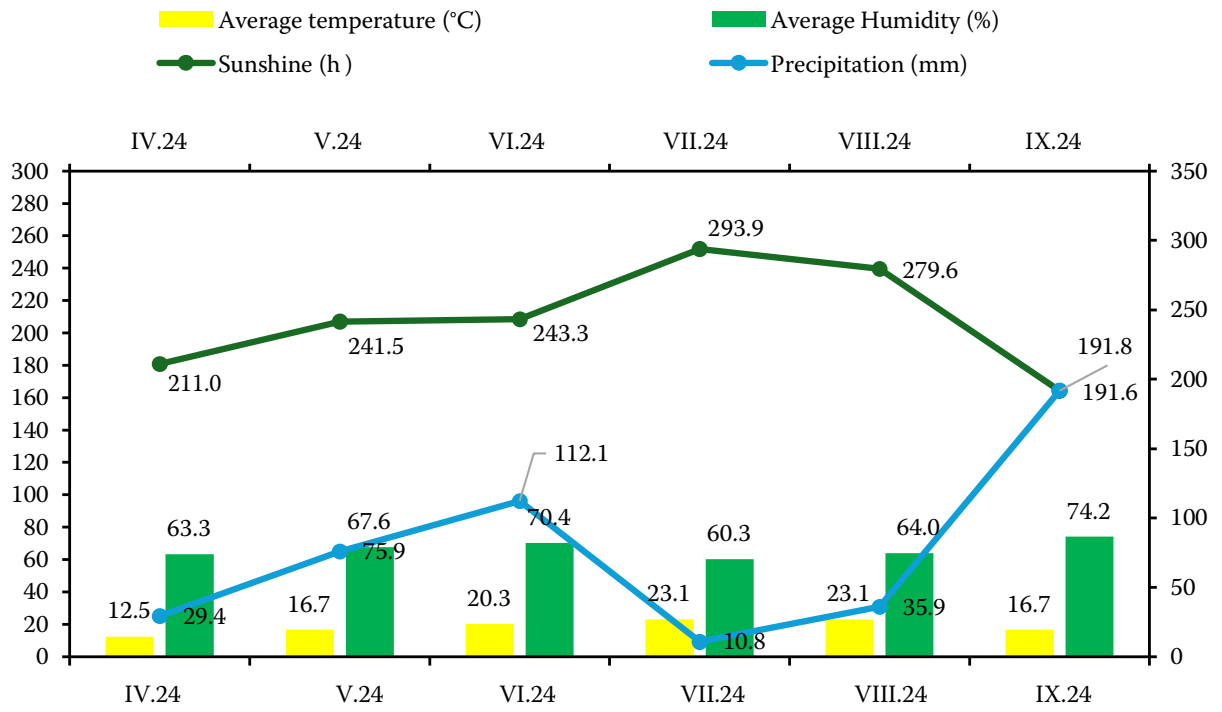

Figure 1. Trends of temperature, humidity, sunshine and precipitation from April to September 2024

tween the plants along the rows, which were 1.5 m apart, under uniform conditions. The soil, mulched with black, non-woven fabric, is sandy-loam and has the following characteristics at 30 cm depth, referred to dry matter: 1.8 mg/kg of ammoniacal nitrogen; 8.44 mg/kg of nitrate nitrogen; 9.71 mg/kg of mineral nitrogen; 283 mg/kg of potassium; 69.2 mg/kg of phosphorus; 291 mg/kg magnesium; 6 770 mg/kg of calcium; CEC, 369 mmol/kg; pH 7.21.

The cultivation practices typical of tomatoes were the following: tying plants to supports, removing side shoots, fertilisation, drip irrigation, and harvesting. The experiment was designed using a complete randomised block design (CRBD) with two treatments, three replications, and three sub-replications. The treatments included: *(i)* a single foliar application of azadirachtin (NeemAzal-T/S at 1% concentration), and *(ii)* a control treatment in which plants were sprayed with just water. In each sub-replication, five plants were selected to sample various plant parts: *(i)* leaf samples from the plant, *(ii)* green fruits from the plant, *(iii)* ripened fruits from the plant, *(iv)* green fruits wrapped in polythene bags from the plant, *(v)* ripened fruits wrapped in polythene bags from the plant. Each sample was analysed individually, and the final residue findings were acquired by pooling the data from each sub-replication. One leaf and two fruits were collected from the chosen plants during the predetermined interval of collections. To avoid direct contact with spray droplets, the fruits of plants 4 and 5 were wrapped in clear polythene bags before being treated with azadirachtin. A key concern addressed in this study was whether azadirachtin, a neem-based biopesticide, acts systemically within the plant or remains localised at the application site. To evaluate this, we used azadirachtin (1%), which was made available under the trade name NeemAzal-T/S (Trifolio-M GmbH, Germany). The application was performed according to the manufacturer's guidelines and applied to the plants at 83-89 BBCH. The azadirachtin was applied as a foliar spray to assess its degradation rate and potential systemic movement. The application was performed using a handheld sprayer to ensure uniform coverage of the foliage. Based on the recommended dosage of 3 L/ha, a concentration of 7.5 mL in 2 L of water was used per treatment. A single spray was applied to 70-day-old fruiting plants to ensure uniform application and facilitate accurate monitoring of the degradation rate without interference from additional sprays. Spraying was conducted in the early morning to minimise degradation due to sunlight and to maximise absorption.

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Table 1. Separation conditions

Column	Arion Polar C18 2.2 µm; 2.1 × 100 mm
Separation temperature	60 °C
Sample injection volume	5 µL
Mobile phase flow rate	0.3 mL/min
Mobile phase A	1% HCOOH in water
Mobile phase B	1% HCOOH in ACN

Table 2. Gradient program

Time (min)	Mobile phase B (in %)
0.00	30
1.50	100
1.51	0
1.99	0
2.00	30

Table 3. Detector settings

Ionisation voltage	3 600 V
Curtain gas (CUR)	45 psig
Collision gas (CAD)	Medium
Nebuliser gas (GS1)	60 psig
Turbo gas (GS2)	45 psig
Desolation temperature (TEM)	450 °C

Azadirachtin analysis

Chemicals. Acetonitrile (ACN), water and formic acid (HCOOH) were of purity for Liquid Chromatography Mass Spectrometry (LC-MS) from supplier VWR International, LLC. Merck KGaA, Darmstadt, Germany, provided the azadirachtin standard.

LC-MS determination of Azadirachtin. The leaves and fruits were harvested two hours after the application of azadirachtin (D0). Furthermore, the samples were collected 1, 2, 4, 6, 8, and 10 days after spraying (DAS) and stored at –20 °C for preservation. Azadirachtin from both leaves and fruits of tomatoes was extracted with acidified acetonitrile, which contained 1% acetic acid. For 1 g of plant material, 1 mL of extraction solvent was used. The extraction was carried out at laboratory temperature in the dark for three days. After that, the liquid portion was centrifuged (3 000 × g; 6 min) and used directly for LC-MS determination.

Instrumentation. The analysis was performed using an ExionLC™ AC binary high-pressure system, which comprises an online degasser, two pumps, an autosampler, a column thermostat, and a control unit. Detection was carried out using a Sciex QTrap 3200 mass spectrometer.

Separation conditions. Separation conditions are shown in Table 1.

Gradient program. Gradient program is shown in Table 2. The total runtime between the two samples was 5 min, with mass spectrometric data acquired between 0.9 and 3.0 min. Azadirachtin quantification was performed using an external standard calibration curve, and the result represents the average of three measurements obtained from different multiple reaction monitoring (MRM) transitions.

Detector settings. The electrospray ionisation (ESI) source was operated in positive ion mode with the parameters shown in Table 3.

The Sciex 3200 QTrap™ mass spectrometer was operated in MRM mode, and the transitions used were defined with the parameters mentioned in Table 4.

Data Analysis

A single foliar spray was followed by careful monitoring of the dissipation of azadirachtin residues on various plant parts, including leaves, green fruits, and ripened fruits. Under validated circumstances, GC-MS was used for quantification. The data associated with time points and treatments were statistically processed using ANOVA with Tibco Statistica software, followed by Fisher's LSD test at $\alpha = 0.05$. The azadirachtin degradation curve in treated samples is displayed in the figures, where

Table 4. Parameters of the transition used in the Sciex 3200 QTrap™ mass spectrometer in MRM mode

Q1	Q3	DT (ms)	Compound name	DP (V)	EP (V)	CEP (V)	CE (V)	CXP (V)
703.3	83	50	Azadirachtin MRM 1	41.0	7.8	20.0	52.6	3.1
703.3	161.1	50	Azadirachtin MRM 2	48.0	7.1	20.1	51.0	3.0
703.3	269.1	50	Azadirachtin MRM 3	50.0	7.1	18.0	50.0	4.0

Q1 and Q3 – the parent and daughter ions; DT – dwell time; mass spectrometer parameters: DP – declustering potential; EP – entrance potential; CEP – collision cell entrance potential; CE – collision energy; CXP – collision cell exit potential

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error bars show the standard error (SE) of the mean concentrations across replicates (n = 3).

RESULTS

Leaves

When azadirachtin was applied to green leaves, the concentration first increased but later decreased significantly. At 0 DAS, the azadirachtin level was around 2.1 mg/kg, with significant variation across the samples. The level fell significantly over the next three days, reaching roughly 1.2 mg/kg at 2 DAS, 0.6 mg/kg at 3 DAS, and almost 0.2 mg/kg by 5 DAS. This represents a degradation of nearly 90% within the first five days after the application. By 9–10 DAS, azadirachtin levels were below the maximum residual limit (MRL) of 0.01 mg/kg. The error bars represent the standard error, which was largest at earlier intervals, indicating variability in early deposition and absorption. The research revealed that time had a statistically significant influence on azadirachtin content in treated samples [$F_{(6,14)}$ = 11.0192, P = 0.0001], but there was no significant change in control samples (Figure 2).

Green Fruits

The azadirachtin concentration peaked at approximately 0.27 mg/kg at 0 DAS, with significant fluctuations as indicated by the broad standard error bars. By 2 DAS, the residue had drastically decreased to around 0.10 mg/kg; by 4 DAS, it had further reduced to about 0.03 mg/kg. Residue levels started to decline at 6 DAS, and by 8 DAS, they had dropped below the MRL of 0.01 mg/kg. A sharp drop in azadirachtin residue was noted throughout the 10 days after azadirachtin was applied to green fruits. Throughout the sample period, the azadirachtin content of control fruits remained consistently low, with no statistically significant variations over time [$F_{(6,14)}$ = n.s.]. On the other hand, over time, the amount of azadirachtin in treated fruits decreased statistically significantly [$F_{(6,14)}$ = 7.9905, P = 0.0007]. The error bars indicate variation in residue levels, particularly in the early periods (Figure 3).

Ripened Fruits

In ripened fruits treated with azadirachtin, the first residue level at 0 DAS was around 0.20 mg/kg, with a significant standard error showing variability across samples. The residual decreased quickly during the first two days, reaching around 0.05 mg/kg. Over the next few days, the residue decreased more gradually. By 6 DAS, the azadirachtin concentration was approaching 0.01 mg/kg MRL, and by 8 to 10 DAS, it had dropped below this level. With no statistically significant change over time, the azadirachtin content of control fruits remained very low [$F_{(6,14)}$ = n.s.]. On the other hand, over time, the amount of azadirachtin in treated fruits decreased statistically significantly [$F_{(6,14)}$ = 5.5635, P = 0.0039]. Early post-treatment days showed significantly wider standard error bars, indicating a considerable degree of initial variation in the distribution of residues across fruits (Figure 4).

Green and ripe-covered fruits

When azadirachtin was applied, both green and mature fruits that were physically covered showed no azadirachtin residues at all sample intervals from 0 to 10 DAS. Azadirachtin levels in both treatment and control samples remained consistently below 0.000 mg/kg, which is far below the MRL of 0.01 mg/kg. There were no discernible effects of treatment or time on residue levels, as indicated by statistical analysis [$F_{(6,14)}$ = n.s. for both groups]. Together, these findings suggest that an effective way to prevent azadirachtin deposition in both green and mature fruit stages is to cover the fruits during spraying.

This absence of residue in covered fruits contrasts sharply with uncovered plant parts, where statistical analyses reveal significant differences in azadirachtin residue dynamics over time and among tissues. The ANOVA revealed three sources of variance: *(i)* azadirachtin treatment (whether or not plants were treated), *(ii)* days after spraying (temporal factor), and *(iii)* the way they interacted (treatment × time). This approach enables us to determine if residue levels are influenced by treatment, time, or their combined impact (Table 5). The ANOVA results indicated that treatment and days after spraying had a statistically significant effect on residue levels in leaves, green fruits, and ripened fruits ($P \leq 0.05$ to $P \leq 0.001$). In contrast, no significant changes were observed in fruits covered with polythene bags ($P > 0.05$), suggesting that there was limited or no systemic movement of azadirachtin into these tissues. The LSD test further highlighted substantial differences in residue concentrations between plant parts, with the high-

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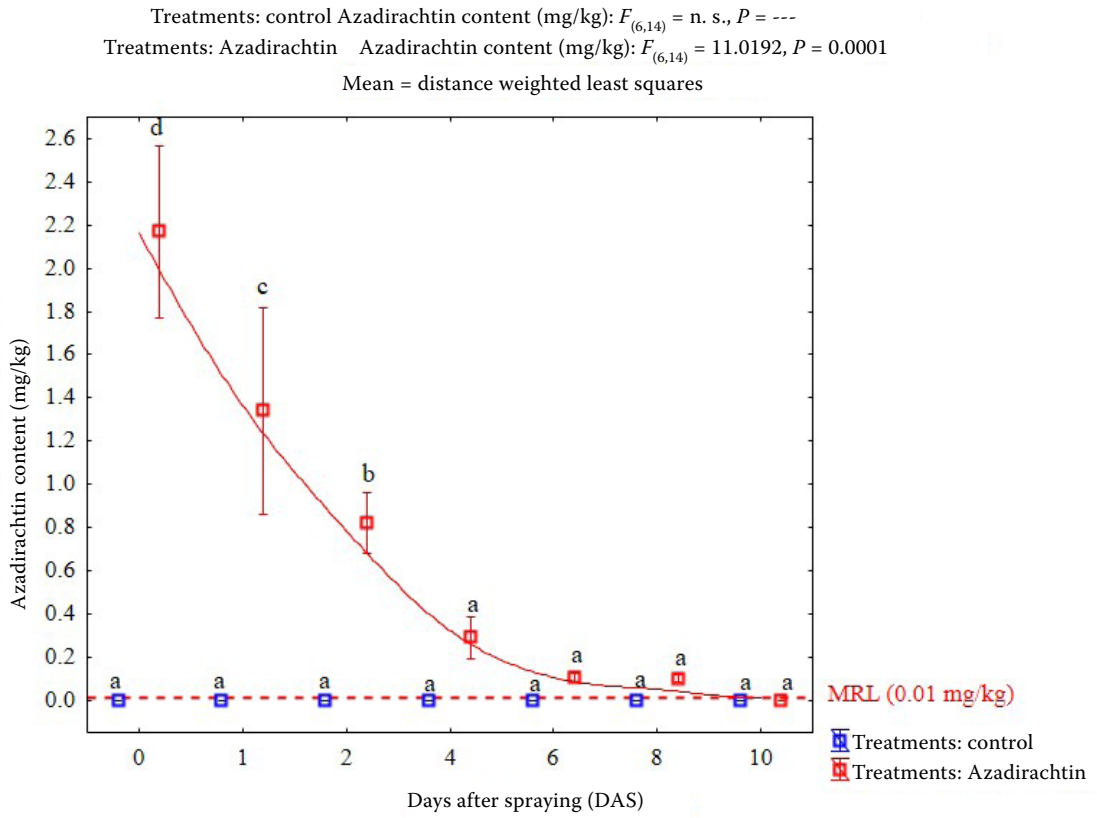

Figure 2. Azadirachtin residue dynamics on leaves post-application (mean values ± SE)

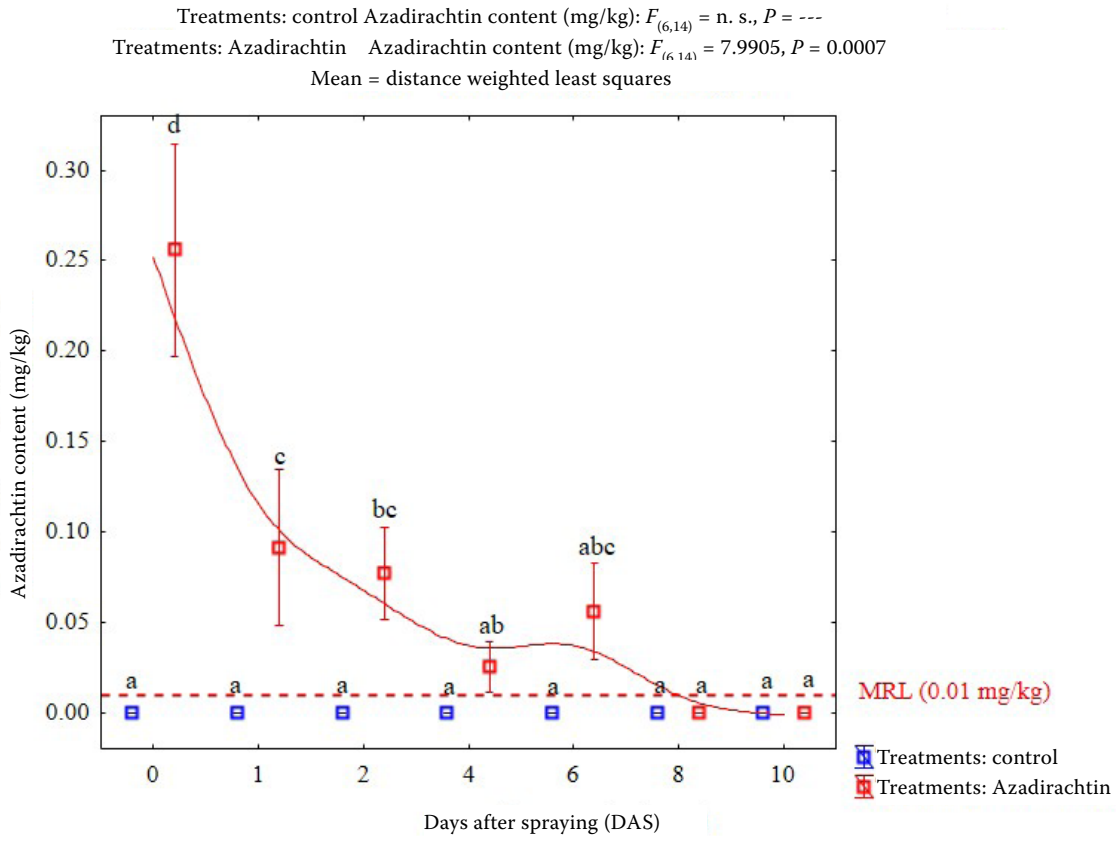

Figure 3. Azadirachtin residue dynamics on green fruits post-application (mean values ± SE)

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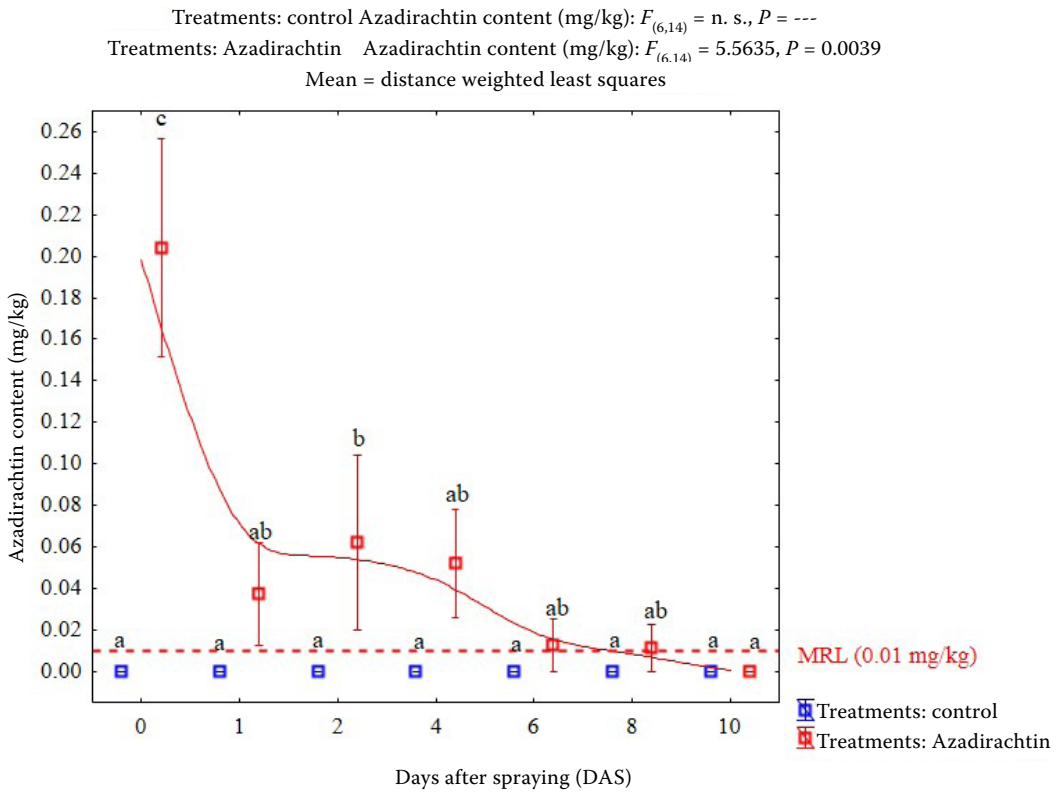

Figure 4. Azadirachtin residue dynamics on ripened fruits post-application (mean values ± SE)

est mean square error (MS = 0.08976) observed in leaves, followed by green fruits (MS = 0.00147) and ripened fruits (MS = 0.00132).

DISCUSSION

The current study aims to determine the permanence of azadirachtin and its possible systemic transport by investigating its residual presence on leaves, green fruits, and ripened fruits after foliar application. During the 10-day observation period, azadirachtin residues in the treatment and control groups were consistently below the MRL of 0.01 mg/kg. These findings will have a significant impact on the use of azadirachtin in integrated pest management (IPM), particularly for crops nearing harvest. Although leaves had the most significant quantities of azadirachtin residues (> 2.5 mg/kg at 0 DAS), green and ripened fruits had much lower residue levels (0.3 mg/kg and 0.22 mg/kg, respectively). This disparity is likely due to structural and physiological variations across plant tissues. Unlike fruits, leaves include stomata, which are small openings that allow foliar-applied chemicals to enter interior tissues (Eichert & Goldbach 2008). Fruits often have fewer or no stomata and are coated with thicker cuticles, which prevent penetration and keep residues on the surface source.

Table 5. Summary of analysis of variance for residues on leaves and fruits

Source of variance	Leaves	Green fruits	Ripened fruits	Green fruits covered	Ripened fruits covered
Azadirachtin treatment	*	***	***	NS	NS
Days after spraying	***	***	***	*	*
Azadirachtin treatment × No. of days after spraying	***	***	***	NS	NS

Differences in residues on leaves and fruits depending on treatment and days after spraying by Fisher's test; non-significant (NS) or significant at $P \leq 0.05$ (*), 0.01 (**), or 0.001 (***), respectively

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Furthermore, the rapid reduction in azadirachtin residues across all plant components (leaves, green fruits, and ripened fruits) during the first few days indicates rapid disintegration, which is facilitated by environmental variables such as sunlight and microbial activity. This finding is consistent with prior research demonstrating azadirachtin's instability in field circumstances (Schmuterer 1990; Isman 2006). Sundaram and Curry (1994) found DT_{50} values of 17–22 h for azadirachtin on fir and oak leaves, indicating that dissipation is strongly influenced by temperature, UV exposure, and the physicochemical properties of plant surfaces – such as the concentration and composition of cuticular wax (Sundaram & Curry 1994; Shaurub et al. 2014). In our experiment, residue levels on all inspected plant parts- such as leaves, green fruits, and ripened fruits were negligible by 8 DAS. By 10 DAS, residues had completely degraded and were no longer detectable. These findings support azadirachtin's transient nature in field settings and imply that its interactions with the epicuticular wax layer may affect the compound's photostability and rate of degradation (Sarais et al. 2009).

The leaves had the highest initial azadirachtin deposition (2.1 mg/kg at 0 DAS), most likely due to their larger surface area, waxy cuticle, and higher stomatal density, which improved adhesion and possibly absorption. Green and ripened fruits, on the other hand, showed lower initial residues (0.27 and 0.20 mg/kg, respectively). This is due to their thicker cuticles, lower stomatal density, and distinct compositions of epicuticular wax, all of which act as barriers to chemical penetration (Eichert & Fernández 2023). These results suggest that leaves exhibited the most significant variability in azadirachtin residues, likely due to more direct exposure and possibly higher surface absorption. In contrast, the lower variability in fruits points to more degradation, consistent patterns, or reduced initial deposition. Residues then rapidly decreased over time in all plant parts, with statistically significant reductions in leaves [$F_{(6,14)} = 11.0192$, $p < 0.0001$], green fruits [$F_{(6,14)} = 7.9905$, $p = 0.0007$], and ripened fruits [$F_{(6,14)} = 5.5635$, $p = 0.0039$] showing consistent dissipation dynamics from photodegradation, enzymatic activity, and environmental exposure. Marín-Sáez et al. (2023) found that azadirachtin breaks down quickly in the field, supporting the compound's low persistence and compliance with food safety regulations(Marín-Sáez et al. 2023).

Additionally, the substantial interaction between treatment and time in uncovered samples, as opposed to non-significant results in covered fruits, suggests that environmental factors—such as sunshine and rainfall—are primary drivers of azadirachtin degradation rather than plant metabolism. These findings are consistent with prior research: Caboni et al. (2006) found that azadirachtin remained surface-bound on strawberries (Caboni et al. 2006), while Sarais et al. (2009) discovered a similar pattern on peaches, with no systemic absorption into the fruit pulp (Sarais et al. 2009). Thus, the non-systemic character of azadirachtin ensures minimal residual levels in edible tissues, thereby contributing to increased food safety. Such phenomena of azadirachtin could be utilised in pesticide residue-free greenhouse tomato production by avoiding ripened fruit zones during spraying.

From a food security and regulatory perspective, azadirachtin's rapid degradation profile provides it a substantial benefit over synthetic pesticides, which are often associated with long-term environmental persistence and potential accumulation (Aktar et al. 2009). Complete residue dissipation before the conventional harvest window improves adherence to zero-residue agricultural regulations, which is especially crucial for organic farming. Thus, the data support azadirachtin's potential as an alternative pest management technique that balances effectiveness, food safety, and environmental sustainability. According to Kilani-Morakchi (2021) and Daraban (2023), the results align with the growing demand for pesticide residue-free vegetables that satisfy customer demands for environmental sustainability and food safety (Kilani-Morakchi et al. 2021; Daraban et al. 2023). Furthermore, hydrolysis and microbial breakdown are the main components of its degradation route, further lowering environmental hazards by producing non-toxic metabolites (Schmuterer 1990). Because of these qualities, azadirachtin is a valuable tool for IPM programs and zero-pesticide-residue technology, which highly values selective pest control and minimal environmental damage.

It is essential to emphasise that the agroclimatic conditions throughout the experimental period (April to September 2024) significantly influenced azadirachtin dissipation dynamics. The study

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was conducted under temperatures ranging from 12.5 °C (April) to 23.1 °C (July and August), relative humidity levels of 29.4–75.9%, and extended periods of sunshine, peaking at 293.9 h in July. Precipitation was variable, with notable rainfall in June (102.1–112.1 mm) and September (191.8 mm), which likely contributed to azadirachtin wash-off and affected degradation rates. These environmental factors, particularly sunshine and rainfall, are known to accelerate azadirachtin breakdown via photolysis and leaching. Consequently, under these conditions, residues were reduced to undetectable levels approximately 10 days after application, especially during summer when solar radiation was strongest, supporting the compound's safe use in crop protection.

However, dissipation may be slower in partially shaded areas of the plant canopy or during cooler months, such as April and September, where lower UV exposure and temperatures can inhibit photodegradation and microbial activity, thereby prolonging azadirachtin's half-life (Mordue & Blackwell 1993). Additionally, its hydrophilic properties can promote leaching and microbial metabolism in humid environments, further minimising persistence on crop surfaces (Pereira et al. 2019). These findings align with previous research, which shows that increased sunshine and warmth accelerate photolytic and microbiological degradation processes (Caboni et al. 2006; Isman 2006). Therefore, while these results confirm azadirachtin's rapid dissipation, generalising them to other regions or growth conditions should be done cautiously, considering local environmental variables.

In addition to climatic factors, plant surface morphology, such as the presence of trichomes, waxy cuticle thickness, and epidermal roughness, may impact azadirachtin retention and breakdown. Highly hydrophobic or waxy surfaces can impede efficient wetting and, consequently, diminish active component deposition. However, thick trichome layers can trap droplets and increase the surface area for degradation processes (Fernández & Eichert 2009).

Azadirachtin's rapid breakdown in open fields demonstrates its environmental safety and applicability for residue-sensitive crops; nonetheless, several important factors necessitate further research. Environmental factors, including temperature, relative humidity, solar radiation, and precipitation, may influence degradation kinetics. Growers must understand that microclimatic factors, including localised shade, surface moisture retention, and sun exposure, can significantly affect residue persistence, even though this study showed a consistent decrease in azadirachtin residue levels under open-field circumstances.

CONCLUSION

This study was intended to investigate the degradation profile of azadirachtin under specific open-field conditions rather than its insecticidal efficacy, which has been thoroughly established in previous research. Based on our findings, azadirachtin degrades rapidly on plant surfaces, with residues falling below detectable levels within 8 to 10 days after a single foliar application. Moreover, it is not transported to fruits via leaves, which may be used in Integrated Pest Management by precise application to avoid ripened fruit zones. Temperature, sunshine, precipitation, and plant surface features had an impact on degradation rates. The outcome of the present study supports the use of azadirachtin in low-residue or pre-harvest applications, particularly in systems that prioritise food safety and environmental sustainability. Future research should concentrate on improving application tactics by investigating the influence of microclimatic variation and leaf or fruit surface morphology in determining degradation kinetics.

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