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A cross-sectional study on occupational exposure to airborne azoxystrobin in tomato greenhouses post-spraying

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Abstract

Introduction: The global demand for year-round vegetable production has intensified the use of pesticides in greenhouse farming, posing potential health risks to agricultural workers. This study investigates the levels of Azoxystrobin, a widely used fungicide, in the air inside a tomato greenhouse following its application, aiming to assess the occupational exposure risks during and after the critical re-entry period.

Methods: Airborne particulate matter and volatile organic compounds (VOCs) were measured at two strategic locations inside the greenhouse: along the external rows (PP1) and the central area (PP2). Sampling was conducted 48 hours post-application (T0) and two months later during the harvesting phase (T1). DustTrak DRX and PhoCheck Tiger devices were used for real-time monitoring of particulate matter and VOCs, respectively. Statistical analyses, including the Wilcoxon signed-rank and Mann-Whitney U tests, were performed to evaluate the differences in concentrations over time and between locations.

Results: At T0, significantly higher concentrations of particulate matter were detected at PP2 compared to PP1, with total dust levels surpassing the 10% Threshold Limit Value (TLV) at PP2. VOC concentrations at the plants (PP3) exceeded exposure limits, placing them in the "high" risk category, while VOC levels further from the plants (PP4) were within the "average" risk range. By T1, both particulate and VOC concentrations had significantly decreased, reducing exposure levels to below the low-risk threshold.

Discussion: The findings underscore the substantial exposure risks associated with re-entry into greenhouses following pesticide application, revealing that elapsed time alone may not be sufficient for ensuring worker safety. Continuous environmental monitoring and strict adherence to personal protective equipment (PPE) protocols are crucial for reducing occupational hazards. Enhanced regulatory guidelines and comprehensive training programs are recommended to improve safety practices in greenhouse environments. Future studies should expand monitoring efforts across various settings to better inform occupational health policies.

Keywords: Chemical risk assessment; chemical risk analysis; greenhouse; particular matter; pesticides; VOCs.

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INTRODUCTION

The introduction of pesticides in agriculture has brought numerous advantages to the agricultural sector and public health, such as eliminating certain diseases (e.g., malaria, yellow fever) from specific territories and achieving greater agricultural productivity. However, pesticides also pose significant potential dangers to human health and the environment. The application methods for different pesticides can vary greatly depending on agricultural contexts, such as the type of crops, soil conditions, seasons, and geographical location. Monitoring pesticide residues in treated crops is essential to ensure food safety and to prevent the bioaccumulation of pesticide residues through the food chain.

In occupational settings, preparing and spraying phytosanitary mixtures are the most hazardous phases for operators' health. During these phases, personal protective equipments (PPEs) are mandatory for all workers. These include full-face or half-face masks with filters to protect against organic vapors, solvents, toxic dust, fumes, and mists; protective coveralls; rubber boots resistant to penetration, permeation, and degradation; safety goggles with good mechanical resistance, watertight and with side covers; and waterproof, mechanically resistant neoprene or nitrile gloves.

A critical issue frequently encountered among workers exposed to chemical risks is resistance to using PPE. Even when employers provide PPE as required by current legislation, workers often neglect to use it due to insufficient information about the risks associated with chemical exposure, leading to a low perception of risk. Additionally, workers using multiple PPE items, such as masks and glasses, often report difficulties in performing their tasks due to interference between the equipment.

Another critical phase involves the return to work in treated areas, which is only allowed after a specified period indicated on the product datasheet. The risk here is associated with the potential presence of pesticide residues in greenhouses, where variable pesticide accumulation and lack of runoff from plants can occur. Therefore, systematic and careful monitoring of such residues is crucial.

Azoxystrobin is one of the most frequently used pesticides. It is a natural antifungal substance produced by saprophytic fungi that prevents the development of other fungal species [1]. The commercial product also contains excipients in low concentrations, such as ethoxylated alcohols, 1,2-

Propanediol, dimethylnaphthalenesulfonate acid polymerized with formaldehyde, and methylnaphthalenesulfonate acid sodium salt [2]. According to the CLP Regulation, its formulation is classified under toxicological and ecotoxicological hazard classes due to its harmful effects on human health and the aquatic environment [3,4]. Once Azoxystrobin application is completed, re-entry to treated areas is permitted after 48 hours [5,6].

This study aims to evaluate the residual chemical exposure risk for workers in a tomato greenhouse treated with Azoxystrobin at two different times: upon worker re-entry and after two months (the time of vegetable collection) [7]. Specifically, this research investigates whether the concentrations of pesticide residues present a significant health risk to workers immediately after re-entry and over a prolonged period, thereby providing critical insights into the effectiveness of current safety protocols and the need for continuous monitoring and enhanced protective measures.

METHODS

Study Design, Target Population, and Sampling

This study was conducted in a tunnel greenhouse with an iron frame, covering an area of 320 square meters (8 meters wide and 40 meters long). The target population consisted of agricultural workers exposed to pesticide residues in the greenhouse environment. The sampling strategy included two different time points: 48 hours after pesticide treatment (T0) and two months after treatment (T1), corresponding to the vegetable harvesting phase. Environmental monitoring was performed at two points within the greenhouse: the external rows (PP1) and the center (PP2).

Study Procedure

Greenhouse Under Observation

The greenhouse under study is equipped with a transparent plastic sheet covering and features a large main entrance for the entry of vehicles and equipment. The door is made of plastic material similar to the rest of the greenhouse. The greenhouse can be completely closed to optimize solar radiation without dispersing heat or opened on the sides to allow air circulation during the crop growth phases. The phytosanitary mixture was distributed via sprayers placed outside the greenhouse through special openings made on the walls.

The first environmental monitoring was carried out 48 hours after the completion of the treatment. The second environmental monitoring was carried out after two months, during the tomato harvesting phase, to consider the possible emission of chemical residues left on the plants during manipulations.

Methods of Monitoring

We used DustTrak DRX (TSI Inc, MN, USA), a laser photometer that measures mass and particle size of particulate, and PhoCheck Tiger (ION Science LTD, UK), a detector for a wide range of VOCs.

- DustTrak DRX: This desktop monitor is a multi-channel, battery-operated, data-logging, light-scattering laser photometer that provides real-time aerosol mass readings and collects gravimetric samples. It uses a sheath air system that isolates the aerosol in the optics chamber to keep the optics clean for improved reliability and low maintenance. It is suitable for both clean and harsh industrial environments, including construction and environmental sites. The DustTrak DRX monitor measures aerosol contaminants such as dust, smoke, fumes, and mists.
- PhoCheck TIGER: This portable gas detector uses photo-ionization technology to detect a

large range of volatile organic compounds (VOCs), which can be dangerous from both a poisoning and explosive perspective. The TIGER uses a photo-ionization detector (PID) to measure gas concentrations. The Health & Safety mode is used to check for conformity with short-term exposure levels (STEL) or time-weighted averages (TWA) specific for particularly hazardous environments (e.g., EH40 in the UK and OSHA in the USA). In this mode, STELs and TWAs are continually calculated and compared to levels set in the instrument's gas table.

Monitoring was performed in the external rows (PP1) and at the center of the greenhouse (PP2) at 48 hours (T0) and after two months (T1). The duration of monitoring was 60 minutes for VOCs and 480 minutes for dust. Data acquisition intervals were set at one value every 48 seconds at T0 and one value every 70 seconds in the central rows, and every 93 seconds in the external rows at T1.

Assessment of Exposure

Environmental monitoring aimed to identify powdery residues and VOCs. Powder forms as a consequence of the evaporation of the liquid fraction and subsequent crystallization of the particulate, while VOCs are products of degradation phenomena under environmental conditions. Powder residues were expressed as mg/m^3 of different fractions (Total, Respirable, PM10, PM2.5, PM1), while VOCs were expressed as mg/m^3.

Applying the evaluation criteria suggested by the UNI EN 689 norm ("Evaluation of occupational exposure"), the applied formula was I=OECLVI = \frac{OEC}{LV}I=LVOEC, where:

- III = Index of the substance
- OECOECOEC = Concentration of professional exposure weighted over eight hours
- LVLVLV = Limit Value

Results greater than one were considered above the limits.

Statistical analyses

Quantitative variables were summarized using means and standard error, with minimum and maximum values indicating the distribution. Non-parametric statistical tests were chosen for their robustness and suitability for non-normally distributed data. Comparisons within the same control point were evaluated using the Wilcoxon signed-rank test, while comparisons between the two control points used the Mann-Whitney U test. A p-value of 0.05 or less was considered statistically significant. All statistical analyses were performed using SPSS® Advanced Statistical TM 13 (2004, Chicago, IL, USA) software package.

The Wilcoxon signed-rank test was chosen for within-group comparisons as it is a non-parametric test that assesses differences in paired samples. It is particularly useful when the data do not follow a normal distribution. The Mann-Whitney U test was used for between-group comparisons due to its ability to handle non-parametric data and compare differences between two independent groups. These tests were applied to ensure the statistical analysis's robustness given the data distribution's non-normality.

Ethical Aspects

This study was conducted following ethical guidelines and received approval from the relevant ethics committee. All workers involved in the study provided informed consent after being fully informed about the study's purpose, procedures, potential risks, and benefits. The data collected were treated with strict confidentiality to protect the privacy and rights of the participants.

Workers were actively involved in the study and were informed about the results and their implications for their health and safety. Information sessions were conducted to educate the workers on the risks associated with pesticide exposure and the importance of using personal protective equipment (PPE). This engagement aimed to enhance the workers' understanding and compliance with safety measures, ultimately contributing to a safer working environment.

RESULTS

The measurements of the different particulate fractions showed statistically significant differences between the two points of the greenhouse, with the highest concentrations in the central points (PP2). At T0, concentrations at PP1 were PM1 0.052±0.003 mg/m³, PM2.5 0.053±0.003 mg/m³, Respirable 0.057±0.003 mg/m³, PM10 0.057±0.003 mg/m³, and Total 0.146±0.010 mg/m³. In contrast, PP2 had higher concentrations: PM1 0.125±0.010 mg/m³, PM2.5 0.127±0.010 mg/m³, Respirable 0.134±0.018 mg/m³, PM10 0.241±0.018 mg/m³, and Total 0.407±0.032 mg/m³ (Figure 1).

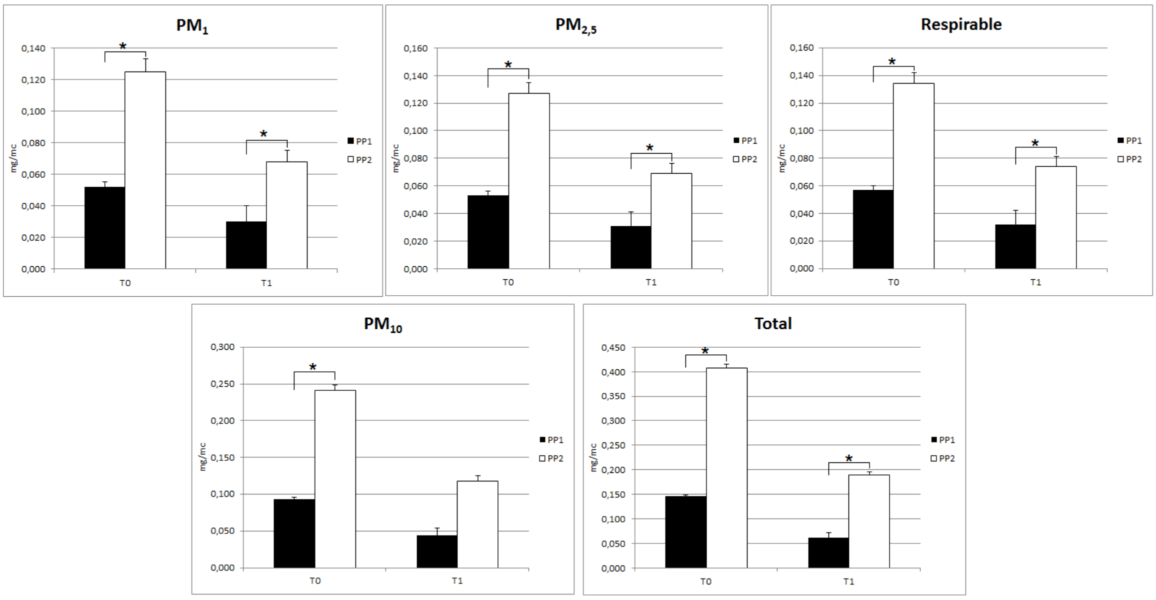
The concentration of particulates decreased significantly from T0 to T1 at both sampling points. For example, the maximum values recorded at PP1 were PM1 0.745/0.136 mg/m³, PM2.5 0.751/0.136 mg/m³, Respirable 0.795/0.0141 mg/m³, PM10 1.480/0.202 mg/m³, and Total 2.570/0.396 mg/m³. At PP2, the maximum values were PM1 2.050/1.150 mg/m³, PM2.5 2.060/1.150 mg/m³, Respirable 2.130/1.200 mg/m³, PM10 3.600/1.630 mg/m³, and Total 5.890/2.242 mg/m³ (Table 1).

Table 1. Concentration (mg/m³) of particulate matter detected in different locations inside the greenhouse at two control times.

Variable	PP1 T0 mean ± SE (min-max)	PP1 T1 mean ± SE (min-max)	PP2 T0 mean ± SE (min-max)	PP2 T1 mean ± SE (min-max)	p-value*
PM1	0.052 ± 0.003 (0.015-0.745)	0.030 ± 0.008 (0.015-0.136)	0.125 ± 0.010 (0.017-2.050)	0.068 ± 0.007 (0.014-1.150)	<0.001
PM2.5	0.053 ± 0.003 (0.016-0.751)	0.031 ± 0.009 (0.016-0.136)	0.127 ± 0.010 (0.017-2.060)	0.069 ± 0.007 (0.015-1.150)	<0.001
Respirable	0.057 ± 0.003 (0.016-0.795)	0.032 ± 0.010 (0.016-0.141)	0.134 ± 0.018 (0.018-2.130)	0.074 ± 0.007 (0.017-1.200)	<0.001
PM10	0.093 ± 0.006 (0.018-1.480)	0.044 ± 0.001 (0.018-0.202)	0.241 ± 0.018 (0.020-3.600)	0.118 ± 0.011 (0.018-1.630)	<0.001
Total	0.146 ± 0.010 (0.018-2.570)	0.061 ± 0.003 (0.018-0.396)	0.407 ± 0.032 (0.020-5.890)	0.189 ± 0.019 (0.018-2.242)	<0.001

Note: $p < 0.001$ Wilcoxon Test T0 vs T1.

Figure 1. Concentration (mg/m³) of particulate fractions measured at T0 and T1 in two points of the greenhouse (PP1, PP2)



Volatile Organic Compounds (VOCs)

Table 2 shows the concentrations of VOCs detected near tomato plants at different distances. PP3 represents the concentration of VOCs detected within 1 cm of the vegetative apparatus, while PP4 represents the concentration measured at approximately 10 cm. The values detected near the plant (PP3) were higher than the exposure limits for the active principle, while concentrations at a greater distance (PP4) were lower.

Table 2. Concentration (mg/m³) of Volatile Organic Compounds (VOCs) detected near tomato plants at different distances.

Variable	PP3 (n=3600)	PP4 (n=3600)	*p-value**
VOCs			
mean ± SE	2.053 ± 0.008	0.044 ± 0.002	<0.001
(min-max)	(0.002-11.830)	(0.001-1.632)	

Note: Mann-Whitney U test PP3 vs PP4.

Risk Index for Particulate Matter

Table 3 highlights the calculated risk index for total dust at the two measurement points inside the greenhouse and at the two control times. There were significant differences between the two points and over time.

Table 3. Comparison of the Index of Substance (I) for Total Dust Calculated at Different Withdrawal Points and Times

Variable	Index of Substance (I) 1° Sampling (T0)	Index of Substance (I) 2° Sampling (T1)	p-value*
PP1	0.0147	0.0054	<0.001
PP2	0.0407	0.0061	<0.001

Note: p < 0.001 Wilcoxon Test T0 vs T1.

Risk Index for VOCs

Table 4 presents the risk index for VOCs calculated at the two measurement points. The values exceeded the limits of the TLV at PP3, while they were below the limits at PP4.

Table 4. Comparison of the Index of Substance (I) for VOCs Calculated at Different Sampling Points

1° Sampling - Parameter: Total VOCs	Withdrawal Point	Unit of Measure	OEC	LV	Index of Substance	p-value*
PP3	mg/m³	2.053	2	1.0265	<0.001	
PP4	mg/m³	0.440	2	0.020	<0.001	

- Mann-Whitney U test PP3 vs PP4.

DISCUSSION

Our analysis revealed that during the first sampling (T0), the concentrations of powdery residues of the phytosanitary mixture were highest at the center of the greenhouse (PP2) and lowest along the external rows (PP1), with statistically significant differences (p < 0.001). Despite ventilation practices, the average concentration of dust did not reach the critical threshold, remaining below 10% of the TLV. However, the maximum value of total dust exceeded the attention threshold (>10% TLV) in both measurement points. Particularly high exposure levels for VOCs were measured near the plants (PP3), where the limit value was significantly exceeded, reaching the "high" risk range. In contrast, the exposure level at PP4 fell within the "average" risk range. The second environmental survey showed a decrease in the concentrations of airborne contaminants at both sampling points over time, reducing exposure levels below the low-risk threshold. These findings indicate that plant management activities at the time of re-entry into the greenhouse represent a significant exposure source for workers. This is in addition to the known toxic effects of Azoxystrobin and its potential for ocular and cutaneous irritation [8-12]. The study underscores the need for appropriate PPE usage to mitigate these risks even in the days following re-entry.

The substance under study has been attributed the following hazard indications: H400 (cat.1) Acute aquatic toxicity; H410 (cat.1) Chronic aquatic toxicity, H302 Harmful if swallowed; H315 Causes skin irritation; H318 Causes severe eye damage; H319 Causes serious eye irritation, and H331 (cat.3) Toxic if inhaled. Regarding exposure control, the occupational exposure limit (8h TWA) for Azoxystrobin is 2.0 mg/m³ for the active ingredient and 10 mg/m³ for particulate matter. For 1,2-Propanediol, the limit is 150 ppm, 470 mg/m³ total (vapors and particulates).

The study confirmed that both the re-entry to the greenhouse (48 hours after antifungal treatment) and the harvesting phase (two months after treatment) pose significant health risks for farmers, exacerbated by the specific microclimatic conditions of the greenhouse soil. The data highlight that merely respecting the return time indicated in technical data sheets does not guarantee adequate safety levels in treated areas, particularly in greenhouse crops. The perception of working safely due to the elapsed time and presumed decomposition or elimination of the chemical product is not entirely accurate. This necessitates the continuous use of specific PPE to ensure worker safety. The use of PPE, however, is heavily influenced by the perception of risk among workers. Younger and less experienced workers tend to use PPE less or incorrectly, while more experienced workers may exhibit overconfidence, leading to underestimation of the risk and inconsistent PPE use.

Enhanced regulations and comprehensive worker training are crucial to mitigate these risks and ensure safer working conditions in greenhouses. Thorough risk assessment and health surveillance are paramount in identifying potential hazards and protecting worker health [13-16].

The study had some limitations, including sample size and scope limited to a single greenhouse, which may not be generalizable to all agricultural settings. Additionally, the study only covered two-time points (T0 and T1), potentially overlooking variations in pesticide residue levels between these periods. External environmental factors such as weather conditions were not controlled, which could affect the concentration of airborne contaminants. However, the study's strengths include detailed and precise monitoring techniques to measure particulate matter and VOCs, practical insights into the risks associated with pesticide use in greenhouses, and non-parametric tests ensuring robust analysis despite potential non-normal distribution of data.

Future research should consider extended monitoring over multiple time points to better understand the dynamics of pesticide residue dissipation and include diverse agricultural settings to generalize the findings. Evaluating the effectiveness of different types of PPE and training programs on improving PPE compliance among workers is also essential. Policymakers should consider revising guidelines to include mandatory continuous environmental monitoring and stricter enforcement of PPE usage. Development and implementation of comprehensive training programs to enhance workers' risk perception and PPE compliance are crucial. Regular health check-ups and surveillance programs should be established for workers exposed to pesticides in greenhouse environments.

This study highlights significant occupational health risks associated with pesticide use in greenhouse environments. The findings emphasize the need for continuous environmental monitoring, stringent PPE usage, and effective training programs to protect the health of agricultural workers. Policymakers and researchers must collaborate to develop and implement strategies that ensure safe working conditions in agricultural settings.

CONCLUSIONS

In conclusion, this study provides critical insights into the occupational health risks associated with pesticide use in greenhouse environments, specifically focusing on Azoxystrobin. The findings demonstrate that both the immediate post-application period and the harvesting phase are high-risk times for workers due to significant exposure to particulate matter and VOCs, particularly in the central areas of the greenhouse. The data underscores the inadequacy of relying solely on elapsed time as a measure of safety, revealing that hazardous levels of residues can persist well beyond the recommended re-entry intervals. The importance of continuous environmental monitoring and strict adherence to PPE protocols is evident, as is the need for improved risk perception and compliance among workers.

This study highlights the necessity for enhanced regulatory guidelines, more comprehensive worker training programs, and further research to ensure safer working conditions in agricultural settings. By addressing these issues, we can better protect the health and safety of those vital to our agricultural industry.

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