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RESEARCH ARTICLE

Farm to table: Residues of different pesticides in tomato and tomato juice – Food safety aspects

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ABSTRACT

During plant cultivation, the pesticides can get into the tissue of vegetables due to crop protection processes, and thus into the food chain. Therefore, they constitute a potential risk to the consumer's health. Depletion of pesticides [spirotetramat (Movento), azoxystrobin and difenoconazole (Amistar Top)] was monitored by testing tomatoes treated individually or simultaneously and tomato juices prepared from the treated tomatoes. The investigations aimed to reveal any kinetic interaction between the compounds tested and changes in their elimination, and thus to assess their compliance with the official Maximum Residue Limits (MRLs). The co-presence of pesticides prolonged the elimination of the individual compounds which reached significantly higher residue levels ($P < 0.0001$) in tomato, especially difenoconazole (45%) and azoxystrobin (50%) on day 8 after treatment that can cause food safety issues to the human consumers. However, the concentrations of pesticides applied alone or simultaneously were found to be below the corresponding MRL values after the withdrawal period in all investigated tomato and tomato juice samples. Accordingly, the investigated pesticides can be safely used simultaneously, their concentrations are in compliance with the legal regulations and thus their concomitant presence does not pose any risk to the consumers' health.

KEYWORDS

spirotetramat, azoxystrobin, difenoconazole, simultaneous application, pesticide residues, vegetables and their processed food

INTRODUCTION

Pesticides – herbicides, insecticides, fungicides, rodenticides – are frequently used for the protection of plants to reduce their damage and contamination and to improve their quality. Among them, herbicides, fungicides and insecticides are sprayed in high amounts worldwide, and many times they (particularly fungicides and insecticides) are applied simultaneously, resulting in toxic interaction (Pimantel et al., 1992; Alexandratos and Bruinsma, 2012; EFSA, 2017; FAO, 2017).

The use of pesticides may pose a risk to the health of the consumer that can be significantly reduced if the given pesticide product is only used according to GAP (Good Agricultural Practice) on authorised cultures with the appropriate technology at the required concentration

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and time. Furthermore, it is important that the official withdrawal period (or preharvest interval) is observed after the application of the product to reduce to, or decrease below, the concentration of the active substance, complying with the legal Maximum Residue Limit (MRL).

In the European Union, the MRLs of authorised pesticidal active substances have been set down by EC Regulation No 396/2005, assuring the safety of raw materials and products of plant origin for the human consumers if the laws and regulations are kept (Commission Regulation, 2005).

One part of pesticidal active substances (and products) can act on the surface of the plants, thus they can be removed by washing, household kitchen preparation (e. g. α-cyhalothrin, chlorothalonil, mancozeb). However, the effectiveness of these processes is variable, depending on the used technology, such as e.g. 10–50–(60)% (washing down with water) or it even may be 79–90% when using heat treatment (microwave, blanching etc.) (Kaushik et al., 2009; Bonnechère et al., 2012; Liang et al., 2012; Bajwa and Sandhu, 2014; Cengiz et al., 2017). Furthermore, their efficacy may be influenced by the physicochemical properties of the substances.

Those active substances and products of pesticides that can absorb from the treated surface and act systemically, and thus can accumulate in the plant (e. g. abamectin, thiamethoxam, azoxystrobin, mefenoxam), pose a higher risk to the human consumers. Edible vegetables and fruits can be considered a potential source of pesticides through consumption by human consumers, particularly when systemic pesticides are used (Claeys et al., 2011; Hlihor et al., 2019). These chemicals can be metabolised in the living plant and excreted from it if the official, authorised withdrawal period of the active substance/product is observed.

The chemicals can be found in the living organisms and the environment not only alone, in themselves, and thus the chemical load can occur in a complex manner while the compounds simultaneously present can interact with one another. This may affect the kinetic movement of the compounds within the organism (toxicokinetic interaction), where a compound can modify the absorption, distribution, metabolism, or excretion of another substance. Thus, the kinetics of the compound within the organism and the levels of their residues may vary significantly and may be present at concentrations above the MRL value. Due to this effect, the active substances are excreted slower, the residual time will be longer, and the official withdrawal period cannot be maintained but must be modified to a longer time interval to allow excretion.

Furthermore, the individual effect of the compounds may be altered or a combined effect may be expected (toxicodynamic interaction), which can be harmful as well.

The combinations of pesticides, particularly if they contain an insecticide, generally increase the toxic effect of the components, or can extend it up to a hundredfold (Thompson, 1996).

Ketoenole derivatives (e. g. spirodiclofen, spiromesifen, spirotetramat) as insecticides inhibit the lipid biosynthesis in the treated insects, resulting in reduced lipid content and ability of adults to reproduce, and inhibition of the growth of younger insects. Spirotetramat (cis-4-(ethoxycarbonyloxy)-8-methoxy-3-(2,5-xylyl)-1-azaspiro[4.5]dec-3-en-2-one, IUPAC) has a systemic insecticidal effect and has particularly high efficacy against the juvenile stages of sucking pests, because it can move upwards and downwards in the xylem and phloem, respectively. It is recommended for the treatment of pome fruits (e. g. apple, peach), stone fruits (e. g. almond, nuts) and vegetables (e. g. cabbage, cauliflower, broccoli, cucurbit, tomato) (Nauen et al., 2006, 2008; Bretschneider et al., 2007; Pesticide Fact Sheet – Spirotetramat, 2008; Marčić et al., 2011).

Strobilurin derivatives (e. g. trifloxystrobin, pyraclostrobin, dimoxystrobin, picostrobin, azoxystrobin) are highly effective to bind to the cytochrome *b* complex III and to block the electron transport chain between cytochrome *b* and *c*1 in the mitochondria, resulting in inhibition of the mitochondrial respiration of fungi. Azoxystrobin (methyl(E)-2-{2[6(2-cyanophenoxy)-pyrimidin-4-yloxy]phenyl}-3-3-methoxy acrylate, IUPAC) has a broad spectrum, and has systemic, translaminar and protective properties. It should always be used in combination with other fungicides exhibiting other modes of action. It is recommended for application to cereals (e. g. wheat, barley, oat), leguminous species (e. g. peas, beans), fruits (e. g. strawberry) and vegetables (e. g. leek, carrot, cabbage, cauliflower, broccoli, lettuce, tomato) (Bartlett et al., 2002; Balba, 2007).

Triazol derivatives (e.g. cyproconazole, metconazole, tebuconazole, difenoconazole) inhibit the demethylation of fungi by blocking 14α-sterol-demethylase thus they reduce the ergosterol synthesis. Furthermore, the 14α-methyl-sterols are accumulated in the plasma membrane of fungi, resulting in its destabilisation and the dysfunction of its enzymes. Difenoconazole (1-[2-[2-chloro-4-(4-chloro-phenoxy)-phenyl]-4-methyl[1,3]dioxolan-2-ylmethyl]-1H-1,2,4-triazole, IUPAC) has broad-spectrum fungicidal action and is applied against fungal diseases of many fruits (e.g. strawberry, honeydew melon), vegetables (e.g. paprika, eggplant, cabbage, cauliflower, broccoli, leek, lettuce, tomato), cereals and other field crops (e.g. sunflower, peas) (Peng et al., 2017; FAO-Difenoconazole-224, 2019).

The aim of this study was to evaluate pesticide residue concentrations of the fungicide Movento (spirotetramat) and the insecticide Amistar Top (azoxystrobin and difenoconazole) applied alone or simultaneously in tomato and in the tomato juice prepared from the treated tomato.

MATERIALS AND METHODS

Cultivation of tomato

Tomato plants were cultivated by hydrocultural method in a greenhouse of the John von Neumann University Faculty of Horticulture and Rural Development (Kecskemét) at three separated parts in order to prevent any cross-contamination.

Soliance F1 type tomatoes with 120–140 g berry weight were planted in rock-wool quilt. The amount and the composition of the nutritive solution were calculated based on the phenotype of the plants and the solution was applied automatically. The temperature and ventilation were regulated automatically, and a shading system and artificial lighting were used.

Treatments

Tomato plants were sprayed with Movento insecticide (spirotetramat, 100 g L^{-1}, CAS No.: 203313-25-1; Bayer CropScience S.A.S.) and/or Amistar Top fungicide (azoxystrobin, 200 g L^{-1}, CAS No.: 131860-33-8; and difenoconazole, 125 g L^{-1}, CAS No.: 119446-68-3; Syngenta AG) alone and simultaneously (FAO-Azoxystrobin-229, 2019; FAO-Difenoconazole-224, 2019; FAO-Spirotetramat-234, 2019). The study design is presented in Table 1.

Based on the license for marketing and use the recommended amount of Movento is 0.75 L ha^{-1} (NFCSO, 2016) for tomato and that of Amistar Top is 1.0 L ha^{-1} (NFCSO, 2017). The spraying solutions were produced by adding 0.9 mL of Movento to 1 L of water, and 1.23 mL of Amistar Top to 1 L of water. The same concentrations of pesticides were used in case of individual and simultaneous treatments, applying four litres in all cases. The plants were sprayed on the morning of the treatment day.

Samplings

Samples of 1 kg of tomato were taken before spray application as control (all groups), on the day of treatments (all groups), on day 2 (all groups), on day 4 (all groups) and on day 8 (Groups II and III) for analytical determination. Each sample was then divided into three individual portions.

Tomato juices were produced from the cultivated tomatoes before the spraying of pesticides and on the day after the withdrawal period (day 4: Movento-treated group, day 8: Amistar Top-treated group and their combined application). Samples of 500 and 500 mL from tomato juices were taken for analysis of pesticide residues from the intermediate and finished product, respectively. Each sample was then divided into three individual portions.

Analytical procedure

Preparation of samples. The tomatoes were cut and homogenised with a stainless steel mixer (Bosch Hausgeräte GmbH, Munich, Germany). Ten-g samples were taken from the homogenised tomatoes and 10 mL from the intermediate and finished product state of tomato juice. The sample was put in a 50-mL polytetrafluoroethylene centrifugation tube and 100 μL of triphenyl phosphate solution (50 μL mL^{-1}) as surrogate standard and caffeine solution (120,000 ng mL^{-1}) as internal standard was pipetted to it. Then, 10 mL acetonitrile was added to the sample, and it was intensively shaken for 1 min. A mixture of 4 g of magnesium sulphate, 1 g of sodium chloride, 1 g of trisodium citrate and 0.5 g of disodium hydrogen citrate, and finally 2 g of magnesium sulphate were added to the sample. After a repeated intensive shaking for 1 min the sample was centrifuged for 5 min at 6,000 rpm and 6 mL of supernatant was transferred into a tube and 750 mg of magnesium sulphate and 125 mg of primary-secondary amine were mixed to it. After that, the solution was shaken for 30 s and centrifuged again for 5 min at 6,000 rpm. Four ml of supernatant was pipetted into a tube and mixed with 5% formic acid, then the sample was evaporated at 45 °C under nitrogen stream to dryness and it was reconstituted with 1 mL acetonitrile containing 0.1% formic acid. After all these steps, the sample was filtered and stored at –20 °C until analysed.

Table 1. Study design

Pesticide formulation	Dose (L ha^{-1})	Concentration (mL L^{-1})	Group		
			I	II	III
Movento	0.75	0.90	+	−	+
Amistar Top	1.00	1.23	−	+	+

Chemicals and standards. Analytical standards of the pesticides were obtained from Sigma Aldrich (Budapest, Hungary). A Phenomenex roQ® QuEChERS extraction kit (Phenomex, USA) including magnesium sulphate, sodium chloride, trisodium citrate and disodium hydrogen citrate was supplied by Gen-Lab Ltd. (Budapest, Hungary). HPLC-grade acetonitrile, ammonium acetate, acetic acid and formic acid were purchased from VWR International (Debrecen, Hungary).

Analytical method. The pesticide content of the sample was measured by UPLC-MS/MS method (Shimadzu LCMS-8030 Plus, Shimadzu Corporation, Kyoto, Japan) using electrospray ion source operating in positive ionisation polarity and multiple reaction monitoring mode. The separation was performed by a chromatography column of Phenomenex Kinetex C18 of 100 × 4.6 mm with 2.6 μm particle size and C18 precolumn (4 × 2 mm) (Gen-Lab Ltd., Budapest, Hungary).

Eluent A contained 50 mM of ammonium acetate dissolved in water and adjusted to pH 5 with acetic acid, and eluent B consisted of 0.1 v/v% formic acid in acetonitrile.

The following parameters were set for measuring: injection volume 10 μL; interface 4.5 kV; temperature: column space 30 °C, sample feeder 5 °C, interface 250 °C, desolvation line 300 °C, heat block 350 °C; flow of nebuliser gas 3 L min^{-1}; flow of dryer gas 15 L min^{-1}. Nitrogen was used for nebuliser and dryer, and argon for collision.

Calibration was carried out in two independent steps in order to cover both the freshly treated samples and those well after the withdrawal period has elapsed. The calibration ranges were between 0.25–250 and 150–2,000 ng mL^{-1} for the first and second case, respectively. The limit of quantitation (LOQ) was 0.25 ng mL^{-1}, and the limit of detection (LOD) was 0.08 ng mL^{-1} for all pesticides investigated.

Data were processed using Shimadzu LabSolutions® software.

Table 2. Results of validation

Compound	Calibration curve parameters – Equation ($y = a \cdot x + b$)* a	b	r**	LOQ (ng mL^{-1})	LOD (ng mL^{-1})	Within-run Precision (%)	Within-run Trueness (%)	Between-run Precision (%)	Between-run Trueness (%)	Recovery (%)
spirotetramat	74.14	−0.20	0.997	0.25	0.07	2.4	108.2	2.1	98.2	92.7
difenoconazole	9.08	0.70	0.997	0.25	0.07	1.2	106.3	3.7	96.2	89.9
azoxystrobin	58.04	0.50	0.999	0.25	0.07	3.3	90.9	1.9	96.4	95.2

* (where 'y' means the peak area ratio between the target compound and the internal standard at the given concentration level; 'x' means the ratio of concentrations)
** regression coefficient
LOQ = Limit of Quantitation, LOD = Limit of Detection

Validation of the method. Before starting the treatments, validation of the selected analytical method was carried out in line with the requirements set by the corresponding EU legislation and scientific guidelines (Commission Decision, 2002; EMEA, 2012).

When checking specificity, 20% of the peak area of the lowest calibration concentration and 5% of the average of peak areas obtained during the calibration were allowed for the target compounds and the internal standard, respectively. No peaks having areas above these limits were observed, which indicates that no matrix-induced false signal can originate from the samples. Good linearity was found in the examined calibration ranges for all studied pesticides with coefficients of determination (r^2) equal or higher than 0.99. Only calibration points fulfilling the preset requirements were considered in determining the equation of the calibration curve during the validation. The LOQ was determined as the lowest point of calibration curves meeting the above requirement. The LOD was calculated for signal-to-noise ratio (S/N) of 3. LOQ values for all analytes were far below the MRL values as regulated by the EU. All within-run and between-run precision and trueness values were within the allowed range (15% for precision and a value between −20% and +10% for trueness). This indicates good repeatability and reliability of the selected method. A summary of the validation results for the studied pesticides can be seen in Table 2. The results of validation proved that the method is suitable for measuring pesticide residues in tomato and tomato juice.

Statistical analysis

The detected concentrations of the active substances after the individual and the combined treatments were compared by two-way ANOVA. Furthermore, the results were evaluated by Microsoft Excel (2019, version: 16.0.6742.2048) software including the percentage comparison of the initial and the final concentrations (at the end of the withdrawal period) of the different pesticidal active substances in tomato and tomato juice (including the heat-treated and non-heat-treated product), and their ratio to the official MRL values.

RESULTS

Pesticide residues in tomato

The concentrations of active substances in tomato samples after individual and simultaneous applications of insecticide and fungicide are presented in Table 3.

Spirotetramat was not detected in the control sample. Its concentration was 208.17 ± 7.15 μg kg^{-1}, 198.09 ± 12.30 μg kg^{-1}, and 180.82 ± 6.91 μg kg^{-1} in the samples on the treatment day, 2 days and 4 days after treatment with Movento, respectively.

Table 3. Concentration of pesticides in tomato (mean ± SD, μg kg^{-1})

Product/Substance	MRL (mg kg^{-1})	Tomato (mean ± SD, μg kg^{-1}) Control	Treatment day	2 days after treatment	4 days after treatment	8 days after treatment
MOVENTO						
spirotetramat	2	<LOQ	208.17 ± 7.15	198.09 ± 12.30	180.82 ± 6.91	NM
AMISTAR TOP						
azoxystrobin	3	<LOQ	941.66 ± 111.67	852.49 ± 98.49	240.09 ± 18.68	157.62 ± 37.61
difenoconazole	2	<LOQ	255.28 ± 57.54	247.07 ± 34.26	195.32 ± 33.24	89.87 ± 24.99
MOVENTO + AMISTAR TOP						
spirotetramat	2	<LOQ	223.17 ± 6.54	181.34 ± 6.89	153.38 ± 20.46	36.57 ± 2.63
azoxystrobin	3	<LOQ	3,547.69 ± 89.75	2,058.28 ± 49.78	1,985.68 ± 186.75	1,768.87 ± 78.02
difenoconazole	2	<LOQ	657.70 ± 65.34	462.51 ± 26.68	365.08 ± 53.17	294.37 ± 12.11

MRL = Maximum Residue Limit, LOQ = Limit of Quantitation, NM = not measured

Azoxystrobin and difenoconazole were not measured in the control tomato samples. The amount of azoxystrobin was 941.66 ± 111.67 μg kg^{-1} on the treatment day followed by 852.49 ± 98.49 μg kg^{-1}, 240.09 ± 18.68 μg kg^{-1}, and 157.62 ± 37.61 μg kg^{-1} on days 2, 4 and 8 after treatment, respectively. The concentration of difenoconazole was determined to be 255.28 ± 57.54 μg kg^{-1}, 247.07 ± 34.26 μg kg^{-1}, 195.32 ± 33.24 μg kg^{-1}, and 89.87 ± 24.99 μg kg^{-1} on the treatment day and on days 2, 4 and 8 after treatment, respectively.

After the simultaneous application of Movento and Amistar Top pesticide products the control samples were free from pesticide residues. The concentration of spirotetramat, azoxystrobin and difenoconazole were 223.17 ± 6.54 μg kg^{-1}, 181.34 ± 6.89 μg kg^{-1}, 153.38 ± 20.46 μg kg^{-1}, 36.57 ± 2.63 μg kg^{-1}; 3,547.69 ± 89.75 μg kg^{-1}, 2,058.28 ± 49.78 μg kg^{-1}, 1,985.68 ± 186.75 μg kg^{-1}, 1,768.87 ± 78.02 μg kg^{-1}; 657.70 ± 65.34 μg kg^{-1}, 462.51 ± 26.68 μg kg^{-1}, 365.08 ± 53.17 μg kg^{-1}, 294.37 ± 12.11 μg kg^{-1} on the day of treatment and on days 2, 4 and 8 after spraying, respectively.

Pesticide residues in tomato juice

Concentrations of active substances in tomato juice samples after individual and concomitant spraying of Movento and Amistar Top are summarised in Table 4.

None of the investigated pesticide products and their active substances applied alone or in combination were detected in the control samples of both non-heated and heat-treated juices.

The concentration of spirotetramat applied alone was 0.52 ± 0.13 μg kg^{-1} and 0.48 ± 0.14 μg kg^{-1} on day 4 after treatment in the non-heated and heat-treated juice product, respectively. Azoxystrobin sprayed individually was not measured in the samples of either type of juice on day 8 after treatment; however, difenoconazole applied alone was detected in the heat-treated juice on day 8 after application (0.32 ± 0.03 μg kg^{-1}).

After combined treatment with Movento and Amistar Top pesticide products, only spirotetramat could be detected in the non-heated and heat-treated tomato juice, at levels of 0.45 ± 0.06 μg kg^{-1} and 0.46 ± 0.04 μg kg^{-1}, respectively.

DISCUSSION

Pesticide residues in tomato

The contamination of vegetables with pesticides during plant protection activities is of overriding importance, and the dissipation of pesticides after spraying is an important factor, particularly if they are applied simultaneously (Omirou et al., 2009; Yang et al., 2020).

The applied pesticides can be quickly degraded in the vegetables, influenced by different factors such as the weather, temperature, the type and properties of the soil, and others (Omirou et al., 2009; Yang et al., 2020). Furthermore, the growth dilution effect can reduce the concentration of pesticides in plants during their growth (FAO, 2017).

The concentrations of spirotetramat in Movento-treated tomatoes exhibit a very slow elimination. On day 4 after treatment (withdrawal period: 3 days), the detected concentration was 180.82 ± 6.91 μg kg^{-1}, which is 86% of the initial amount (208.17 ± 7.15 μg kg^{-1}) measured on the day of application. However, this level is only 9% of the official MRL value (2 mg kg^{-1}).

Pesticide residues after single and concomitant use in juicy fruits and vegetables for human consumption were studied by several researchers.

Mango was treated with the combination of spirotetramat- and imidacloprid-containing pesticides, and the detected concentration of spirotetramat (327 μg kg^{-1}) was below the MRL on the day after treatment. The residue of spirotetramat was reduced by 20% and 80% on day 1 and day 7 after application, respectively. If spirotetramat was used in a double dose, its elimination showed a similar tendency (day 1: 22.7%, day 7: 71%, day 10: 100%). Ripe mangoes with or without peel were free from spirotetramat at harvesting. Based on these data, depletion of spirotetramat from mango was as quick as in tomato. Thus, by the time the fruit and/or vegetable were delivered to the consumer,

Table 4. Concentration of pesticides in tomato juice (mean ± SD, μg kg^{-1})

Product/Substance	MRL (mg kg^{-1})	Tomato juice (mean ± SD, μg kg^{-1})					
		Control		4 days after treatment		8 days after treatment	
		non-heat-treated	heat-treated	non-heat-treated	heat-treated	non-heat-treated	heat-treated
MOVENTO							
spirotetramat	2	<LOQ	<LOQ	0.52 ± 0.13	0.48 ± 0.14	NM	NM
AMISTAR TOP							
azoxystrobin	3	<LOQ	<LOQ	NM	NM	<LOQ	<LOQ
difenoconazole	2	<LOQ	<LOQ	NM	NM	<LOQ	0.32 ± 0.03
MOVENTO + AMISTAR TOP							
spirotetramat	2	<LOQ	<LOQ	NM	NM	0.45 ± 0.06	0.46 ± 0.04
azoxystrobin	3	<LOQ	<LOQ	NM	NM	<LOQ	<LOQ
difenoconazole	2	<LOQ	<LOQ	NM	NM	<LOQ	<LOQ

MRL = Maximum Residue Limit, LOQ = Limit of Quantitation, NM = not measured the residue level was below the detection limit (50 μg kg^{-1}) (Mohapatra et al., 2012).

Spirotetramat was sprayed on citrus fruits against insects and its residues, including its metabolite (20–400 μg kg^{-1}), were below the MRL (1 mg kg^{-1}) after the withdrawal period (Zhang et al., 2017).

During the cultivation of cotton, the combination of spirotetramat and imidacloprid was applied as an insecticide in India; however, their residues were not detected in the cottonseed at harvesting (Pandiselvi et al., 2010).

After treatment with Amistar Top (containing azoxystrobin and difenoconazole) the concentration of azoxystrobin measured in tomato on the day of treatment (941.66 ± 11.67 μg kg^{-1}) was reduced to 17% of the initial value by day 8 after treatment (withdrawal period: 7 days), which is only 5% of the official MRL (3 mg kg^{-1}).

The concentration of difenoconazole showed a slower elimination in tomato; 36% of the initial amount (255.28 ± 57.54 μg kg^{-1}) could still be detected on day 8 after treatment (89.87 ± 24.99 μg kg^{-1}). However, that level was also well below the MRL (4%; 2 mg kg^{-1}).

A similar tendency was noted by Lin et al. (2022). The concentration of the nematicide fosthiazate gradually decreased during the preharvest interval (PHI) of 14 days in tomatoes (0.056–0.058 mg kg^{-1}) and cherry tomatoes (0.135–0.159 mg kg^{-1}). Further reduction was recorded at a PHI of 21 days (tomato: 0.032–0.033 mg kg^{-1}; cherry tomato: 0.043–0.046 mg kg^{-1}). Residues of fosthiazate could not be measured in tomatoes and cherry tomatoes at 28 days of PHI (Lin et al., 2022).

Similarly, the average residue concentrations of the fungicide iprodione and the insecticide thiacloprid applied alone to tomatoes gradually decreased (iprodione: from 1.71 ± 0.12 to 0.42 ± 0.04 mg kg^{-1}; thiacloprid: from 0.74 ± 0.03 to 0.07 ± 0.01 mg kg^{-1}) to values below the official EU MRL (iprodione: 5 mg kg^{-1}; thiacloprid: 0.5 mg kg^{-1}) during the sampling period (0.125 mg kg^{-1} at 20 days) (Omirou et al., 2009).

The concentration of azoxystrobin residues was below the officially set MRL value (15 mg kg^{-1}) on lettuce in a Spanish investigation (Itoiz et al., 2012).

The maximum concentration of azoxystrobin was 1,870 μg kg^{-1} on the treatment day in raw zucchini and then it decreased below the official MRL (1 mg kg^{-1}) 2 days after application, showing a similar decreasing depletion tendency as in tomato (Aguilera et al., 2012).

Rani et al. (2013) reported that the residues of other pesticides (e.g. chlorpyriphos) were below the MRL on day 0 (155.0 ± 0.002 μg kg^{-1}) in tomato fruits.

After the simultaneous application of Movento and Amistar Top, the depletion of spirotetramat exhibits a similar tendency in tomato as in case of individual treatment. Its concentration was reduced to 16% (36.57 ± 2.63 μg kg^{-1}) of the initial amount (223.17 ± 6.54 μg kg^{-1}) by day 8 after application. However, there was no significant difference between individual and simultaneous treatment ($P = 0.0700$).

The concentration of azoxystrobin (1,768.87 ± 78.02 μg kg^{-1}) and difenoconazole (294.37 ± 12.11 μg kg^{-1}) in tomato was found to be statistically ($P < 0.0001$) higher at all sampling times than in case of their individual application. On day 8 after treatment, their concentration was 50% (azoxystrobin) and 45% (difenoconazole) of the initial levels, respectively.

These results indicate that the depletion of azoxystrobin and difenoconazole from tomato was significantly delayed ($P < 0.0001$) due to the combined use of Movento and Amistar Top.

However, Li et al. (2016) stated that the combined use of the fungicides carbendazim and diethofencarb resulted in lower residues than the MRL one day after spraying in tomato.

Abd-Elhaleem (2020) investigated the concentrations of different pesticides in tomato and its products (tomato paste, ketchup). It was stated that the amount and frequency of the measured residual pesticides were higher in tomato than in its products. Generally, the concentrations of detected pesticides (buprofezin, carbendazim, cypermethrin, flubendiamide, iprodione, pyrimethanil, tebuconazole, boscalid) were below the EU MRL values; however, the presence of multiple residues can pose a potential risk to consumers, particularly to children and pregnant women.

However, Tripathy et al. (2021) stated that the combined use of iprovalicarb and propineb in tomato does not result in a potential health risk to consumers. Their residues were generally found to be well below the official MRL values.

Soydan et al. (2021) evaluated the presence of pesticide residues in different fruits and vegetables including tomato. Pesticides were detected in about 50% of tomato samples but were below the official MRL values. Generally, 11.6% of the samples contained multiple pesticide residues exceeding the MRL, including 6 out of 44 tomato samples (4 samples: 2 pesticides; 2 samples: 3 pesticides).

Pesticide residues in tomato juice

Spirotetramat was detected in non-heat-treated (0.52 ± 0.13 μg kg^{-1}) and heat-treated (0.48 ± 0.14 μg kg^{-1}) tomato juices in almost the same concentrations on day 4 after treatment. Its concentration was not influenced by the heat treatment, and it was below the MRL value (2 mg kg^{-1}) in both the non-heat-treated (0.026% of the MRL) and the heat-treated samples (0.024% of the MRL).

The concentration of azoxystrobin in both heat-treated and non-treated tomato juice samples was below the LOQ on day 8 after the application of Amistar Top.

However, difenoconazole could still be detected (0.32 ± 0.03 μg kg^{-1}) in the heat-treated sample on the same day, but it was below the LOQ in the non-heat-treated tomato juice; however, its detected concentration was 0.016% of the MRL value (2 mg kg^{-1}).

The concentration of azoxystrobin in zucchini was not reduced by boiling (Aguilera et al., 2012), but Rani et al. (2013) reported that the residue of chlorpyriphos was decreased by different processing steps including boiling.

Similarly, the residue of pesticides (boscalid, mancozeb, propamocarb) was reduced by heat treatment (blanching, boiling, sterilisation) in spinach by a range of 50–95%. However, the concentration of deltamethrin was increased after boiling (from 157.0 ± 2.0 μg kg^{-1} to 206.0 ± 1.0 μg kg^{-1}) (Bonnechère et al., 2012). Reduction of residue levels in fruits and vegetables by boiling and juicing was also described by Keikotlhaile et al. (2010).

The concentrations of boscalid (0.03 mg kg^{-1}) and carbendazim (0.01 mg kg^{-1}) in tomato paste, and of cypermethrin (0.02 mg kg^{-1}) and pyrimethanil (0.01 mg kg^{-1}) in ketchup were below the EU MRL values, such as 3, 0.3, 0.5 and 1 mg kg^{-1}, respectively (Abd-Elhaleem, 2020).

After the simultaneous application of Movento and Amistar Top, spirotetramat was detected in both the non-heat-treated (0.45 ± 0.06 μg kg^{-1}) and the heat-treated (0.46 ± 0.04 μg kg^{-1}) tomato juice samples, but its concentrations were below the MRL value in both cases. The concentrations of azoxystrobin and difenoconazole were found to be below the LOQ.

Kong et al. (2012a) described that the homogenisation and sterilisation process had little effect on the removal of difenoconazole from tomato. A similar effect was reported by Han et al. (2013) on the fate of chlorpyriphos and its metabolite in tomato and tomato products. However, due to simmer preparation a twofold increase of difenoconazole (76%) was detected in tomato puree (Kong et al., 2012a).

Kong et al. (2012b) described that the residue level of tebuconazole was higher in apple peel and core than in the pulp, and it was concentrated in apple pomace.

Similar results were obtained during the production of apple and cherry juice with the reduction of residues of different pesticides by 78–100% (azinphos-ethyl, chlorpyriphos, fenvalerate, methomyl) and 70–90% (chlorpyriphos, fenamirol), respectively (Zabik et al., 2000; Hadzhikinova et al., 2006).

The following conclusions can be drawn from this study. Raw tomato and tomato products (e.g. tomato juice) are popular raw and processed foods. Therefore, it is important to have information on the possible interaction of pesticides applied simultaneously during cultivation and the resulting concentrations in the raw and finished products provided to human consumers.

It was found that the depletion of azoxystrobin and difenoconazole was much slower in tomato samples after the simultaneous use of the insecticide and fungicide product. A similar tendency could be observed with spirotetramat, as well. However, in each case, the detected concentrations were below the MRL values at the end of the withdrawal period.

The pesticide residues present in the tomato juice samples prepared from tomato taken at the end of the withdrawal period were lower than 1 μg kg^{-1} after both individual and concomitant spraying of raw tomato.

In all investigated samples of tomato and tomato juice, the pesticide residues were below the MRL value, but their co-presence prolonged the depletion dynamics of the individual compounds. Overall, the individual and the simultaneous applications of the pesticides tested were safe and compliant with the regulations, and thus they do not pose any significant hazard to the consumer.

However, the combination products of pesticides and/or their combined use, and thus the multiple pesticide residues confirmed by various researchers, can pose a potential dietary risk to human consumers.

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