

## EFFECT OF PESTICIDES ON THE NUTRITIONAL QUALITY OF CULTIVATED SPICE PAPRIKA

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### Abstract

*Spice paprika, including different species such as bell pepper and chili, is the second-largest spice commodity worldwide. It has varying significance in the European Union (EU) countries from a culinary, economic, and socio-cultural points of view. Due to the high pressure of pests and intensive plant protection, food safety aspects related to mycotoxins and pesticide residues have come to the fore. Currently, there are 41 active ingredients registered in the EU for chemical treatments in paprika cultivation. Pesticides can affect the quality of spice paprika. High dosages of pesticide treatment resulted in lowered levels of tocopherols (up to 13%) and carotenoids (up to 16%). Some of the aims of the European Green Deal are “50% reduction in use and risk of chemical pesticides”, “50% reduction in the use of more hazardous pesticides” and “at least 25% of the EU’s agricultural land under organic farming”. The present collaboration within the Erasmus+ Hort4EUGreen project supports the dissemination of pesticide-free and organic farming knowledge, doubled by knowledge on nutritional quality of horticultural products and urban horticulture, for students, farmers and other interested citizens. This is done open access, via the hortgreen.com educational platform, that aims at becoming a long-term training hub enhancing theoretical and practical skills of horticulture specialists to better address the demands of the European Green Deal.*

**Key words:** *spice paprika, chili, active ingredients, pesticide residues, MRL.*

### INTRODUCTION

Spice paprika, a mix of different species and cultivars such as bell pepper and chili, is the second-largest spice commodity worldwide after black pepper, both in terms of production and trade value (Lakner et al., 2018). In 2019 the total production of peppers in the European Union (EU) was 2.580 thousand tons, while 176 thousand tons were imported from non-EU countries (Fruit Logistica, 2022).

Spice paprika plays different roles in the EU countries, being a market-leading commodity in Hungary and Spain (CBI, 2022). In Hungary, maintaining the quality of spice paprika as a “*Hungaricum*” product is essential. In 2020 the total production of Hungary was more than 15 thousand tons (KSH, 2022). Peppers have a special role in Turkish cuisine. The cultivation started hundreds of years before, when the

ottoman Turks took the pepper from the Portuguese ships carrying peppers from Spain to Arabia. In Romania, the pepper crop ranks fourth, after cabbage, tomatoes and onion, in terms of production (INS, 2022). At world level, in 2018, Romania ranked 15 in the top of world pepper producers, with 229662 tons while Türkiye ranked third, with 2554974 tones, in the same year (Agrostandard, 2020). In Bulgaria, pepper has a significant economic and socio-cultural importance, as across the Balkan Peninsula, due to the favourable climate that favours pepper production.

Due to the high pressure of pests, food safety aspects related to mycotoxins and pesticide residues, and the influence of these contaminations on the nutritional quality of spice paprika are emphasized. The European Green Deal approved in 2020 is a set of policy initiatives by the European Commission to

overcome climate change and reduce environmental degradation, phenomena which are global existential threats to Europe and the world (European Commission, 2022a). Representing the intended meaning of the “from Farm to Fork” concept, the European Green Deal envisages the reduction of the use of pesticides, fertilizers, and antimicrobials in all agricultural practices including farming within all EU-member countries (European Commission, 2022b).

In addition, an increased proportion of organic production is also targeted by the European Green Deal via an increase of the areas involved in organic farming to 25% of the total cultivated area, by 2030 (European Commission, 2022b). Thus, organic cultivation of spice paprika (with no synthetic pesticides applied and eco-friendly cultivations technologies applied) is also promoted.

Our paper overviews the status of spice paprika from the aspect of food and environmental safety. In particular, pesticide residues and other organic microcontaminants occurring in spice paprika, as well as the effect of heavy pesticide applications on spice paprika quality (contamination and levels of bioactive substances) are reviewed. Our summary relates to the Erasmus+ Hort4EUGreen project, a Strategic Partnerships between Hungary, Romania, Bulgaria, and Turkey.

## **PESTICIDES AUTHORIZED IN THE EUROPEAN UNION FOR SPICE PAPRIKA**

The vast majority of spice paprika is cultivated under intensive agricultural practices worldwide. This is justified partly because such production is carried out under monoculture conditions, and partly because treatment with agrochemicals (plant protection products, PPPs, pesticides) is considered to be the most time- and labour-effective means of pest (mostly insect pest) control under such conditions.

Nonetheless, intensive production may result in two disadvantages: the occurrence of pesticide residues and possible alterations in the (bio) chemical composition of the produce due to the chemical stress exerted by the agrochemical treatment. Even though pesticide registration utilises increasing rigor in the authorisation of

new pesticides and in the withdrawal of obsolete ones, the number of allowed pesticide active ingredients is still high, requiring complex pesticide residue and other organic microcontaminant monitoring throughout the entire production process.

Currently, 41 active ingredients are registered in the EU for pest control in paprika cultivations (European Commission, 2022c). The authorized active substances are mainly insecticides (16), and an additional 12, seven, and seven compounds as fungicides, herbicides, and soil disinfectants, respectively, have an approved status.

The control of insects in paprika cultivation is an essential part of chemical plant protection. In recent years, the application of hazardous insecticides such as chlorinated hydrocarbons, organophosphates, and neonicotinoids (except for acetamiprid) for pest control has been reduced or banned (MacBean, 2012; European Commission, 2013), but a range of pyrethroids dominantly remained in use. As in other crops, fungal diseases of paprika cultivation can also result in yield loss, so the application of fungicide formulations is required in intensive cultivation. The most used fungicides are inorganic sulphur and copper compounds (these are approved in organic farming, too), and additional systemic triazoles (e.g., tebuconazole). Various herbicide active ingredients are authorized for weed control in paprika cultivation. The most frequently used herbicide compounds are pendimethalin, napropamide, and chlomazone (MacBean, 2012). However, paprika species are relatively sensitive to herbicides, and generally, weeds are removed mechanically, although naproxamide and glyphosate are often applied against weeds in a pre-emergence setup, before the plantation.

The sustainable agricultural production and eco-friendly pest control of paprika can be supported and have been claimed to be solvable by the use of botanical insecticides (e.g., extract of neem or garlic) and jasmonic acid or with the extraction of earthworm excretory substances (e.g., Vermiwash) (George et al., 2007; Mondal & Mondal, 2012; Awang et al., 2015).

PPPs are regulated by Regulation (EC) 1107/2009 in the EU (European Parliament and

Council, 2009). Of the four mentioned and cooperating countries in the Erasmus+ Hort4EU Green project, three are the member states of the EU, so there appear no differences in the registered active ingredients among them, because active ingredients are authorized at the level of the EU. The pesticide formulation and their uses in various crop cultures, however, are regulated by the member states on their territory under the corresponding European regulations. The authorization of pesticide active ingredients is performed based on a scientific evidence-based risk assessment conducted by the European Food Safety Authority (EFSA). If active ingredients are classified as teratogenic, carcinogenic, or persistent substances, then they cannot be approved for use in plant production products (European Parliament and Council, 2008). Also, if their occurring detrimental effects show non-monotonous dose response, the assessment is hazard-based (and not risk-based).

#### **FOOD SAFETY ASPECTS OF PESTICIDE RESIDUES IN SPICE PAPRIKA**

Nowadays, the intensive agricultural practice relies on different agrochemicals that may exert various possible hazardous effects on food safety, as their residues may appear in crops and agricultural products. Environmental and food safety of spices (e.g., spice paprika) used for culinary purposes and for flavouring in food production, receive comparatively low attention due to the low volume of spice trade networks. In general, contamination surveys of spices assess the microbial impurities or mycotoxins, although residues of pesticides are also prevalent chemical contaminants in *Capsicum* species, including spice paprika and chili (Klátyik et al., 2017). However, in pesticide monitoring programs, more attention is being given to spices and other dried foods because due to their low water content, the level of pesticides is possibly higher (Seo et al., 2013; Galani et al., 2021).

The application of pesticide active ingredients and their formulated PPPs can affect the quality of spice paprika in two ways: via the possible emergence of pesticide residues as environmental contaminants on the quality of

the paprika product and also by affecting the bioactive component content of paprika, due to illegal use of pesticides (e.g., the use of not authorized pesticide formulations), incorrect application of chemical compounds to given crops or improper circumstances of harvesting and storage (e.g., pesticide treatment during storage, harvest before the end of the official waiting period of the last pesticide application). The adverse effects and even phytotoxicity of chemical PPPs on physiological parameters of plants (e.g., enzyme activities, photosynthesis) are known, particularly at high dosages of pesticides (Mitra & Raghu, 1998a; Cali & Candan, 2009; Shakir et al., 2016). DDT and its residues result in a significant reduction in the growth and yield of chili (Mitra & Raghu, 1998b). According to the results of contamination surveys, the highest level of methoxychlor, alachlor, and  $\beta$ -HCH in chili pepper was 7.25 mg/kg, 5.81 mg/kg, and 1.56 mg/kg, respectively, where 58.9% of the analysed chili peppers was contaminated (Galani et al., 2021), and the concentration of p,p'-DDE residues was 0.12 mg/kg in chili peppers (Oyeyiola et al., 2017).

#### **PARALLEL ASSESSMENT OF PESTICIDE RESIDUES AND PRODUCT COMPOSITION OF SPICE PAPRIKA UNDER AGROCHEMICAL PEST CONTROL**

In our semi-field modelling study (Mörtl et al., 2018), an intensively cultivated spice paprika growing site was systematically treated with pesticide premixes at several dosage levels. Exposures to pesticides were assessed partly by monitoring persisting concentrations of pesticide residues in the soil of the cultivation plot, and partly by determining occurring pesticide levels in the produce.

Possible environmental exposure to persisting pesticide residues was characterised by carrying out routine residues in agricultural soils and surface waters in Hungary. During these analyses, the presence of chlorpyrifos, tefluthrin, trifluralin, and DDT with its metabolites (DDD and DDE) was proved in the investigated soil samples. Furthermore, atrazine, diazinon, and metolachlor were detected in some soil samples, although under

the limit of quantification (LOQ). In contrast, pesticide residues as soil contaminants were not detected in soil samples originated from ecological farming. In half of the collected surface water samples, trifluralin was detected in the range of 0.01-0.03 mg/l (Székács et al., 2015; Klátyik et al., 2017). According to the physical and chemical properties of chemical compounds used for plant protection, the residues of persistent compounds can be detected in various environmental matrices for a long time after the application of pesticides.

Thus, the measurement of pesticide residues was performed in various paprika fruit and environmental samples originated from fields under intensive cultivation in Hungary. During our measurements, in the investigated paprika samples, pesticide residues were not detected.

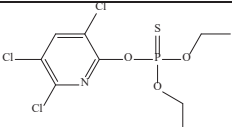
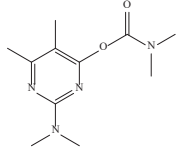
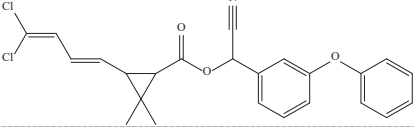
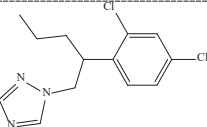
Spice paprika represents a rather difficult sample matrix for analytical measurements because it consists of a complex plant matrix, as it is rich in essential oils and red carotenoids, in addition to high lipid content and alkaloids like capsaicin (Klátyik et al., 2017).

During the food processing, treatment, and storage, bioactive components in spice paprika degraded partially. The stability of  $\beta$ -carotene, as well as red and yellow xanthophylls is highly affected by heat treatments (Krammer et

al., 2001). Decreased levels of  $\alpha$ -tocopherol, ascorbic acid, and red colour content were reported during the processing of red pepper (*C. annuum* L.) (Carvajal et al., 1998). The level of bioactive components, including carotenoids, is highly affected by environmental stressors, including the residues of chemical compounds (Márkus et al., 1999; Havaux, 2014).

In the modelling cultivation study (Mörthl et al., 2018), the experimental design aimed to reveal, whether treatment of spice paprika with various levels of authorized pesticides under semi-field cultivation conditions results in severe occurrence of pesticide residues, and whether it causes alterations in the composition of beneficial bioactive substance of the produce. During our investigation, the effects of pesticide treatments were investigated using a pesticide mixture (three insecticides: pirimicarb, cypermethrin, chlorpyrifos; and a fungicide: penconazole), applied 1-3 times at different dosages, where the level of pesticide residues and the bioactive component content were measured in red pepper and processed spice paprika samples. The biochemical classes of pesticide active ingredients applied in the cultivation experiment are listed in Table 1.

Table 1. The pesticide active ingredients applied in the modelling spice paprika cultivation study

| Pesticide type | Active ingredient | Chemical structure  | Mode of action   | Formulated PPP <sup>1</sup> |
|----------------|-------------------|---|--|-----------------------------|
|                | chlorpyrifos      |  | Inhibition of acetyl cholinesterase in the synaptic gaps between neurons | Cyren EC                    |
| Insecticide    | pirimicarb        |  |  | Pyrimor 50 WG               |
|                | cypermethrin      |  | Interaction with voltage-gated sodium channels in neurons                | Cyperkill 25 EC             |
| Fungicide      | penconazole       |  | Inhibition of ergosterol biosynthesis                                    | Topas 100 EC                |

<sup>1</sup>PPP: plant protection product

The insecticide active ingredients belonged to biochemical classes acting on the insect nervous system by inhibiting the cleavage enzyme of the neural mediator acetylcholine (acetyl cholinesterase inhibitors: carbamate pirimicarb and organophosphate chlorpyrifos), as well as a voltage-gated sodium channel effector (pyrethroid cypermethrin). The applied fungicide is an ergosterol biosynthesis inhibitor (triazole penconazole).

Applied pesticide dosages represented low, average, high and extra high levels of PPP use compared to real agricultural field application rates. Thus, the experiments modelled realistic field situations, and the chemicals stress caused by these agrochemicals is similar or close to those that can occur in routine and common agricultural practices.

After the extraction of the samples (Figure 1), the limits of detection (LODs) of the analytical method for pesticide residues using gas chromatography coupled with mass spectrometry (GC-MS) are shown in Table 2. These LODs indicate that the target substances are detectable in the low ng/g range.

Table 2. Analytical limits of detection (LODs) of the pesticide active ingredients applied

| Pesticide active ingredient | M <sup>-1</sup> | LOD (µg/g) |
|-----------------------------|-----------------|------------|
| chlorpyrifos                | 314             | 0.005      |
| pirimicarb                  | 166             | 0.005      |
| cypermethrin                | 163 + 181       | 0.030      |
| penconazole                 | 248             | 0.010      |

<sup>1</sup>Characteristic quantification molecule ions in mass spectrometry

Residues of pesticide active ingredients administered to the crop readily occurred in the produce. The insecticide active ingredient chlorpyrifos was readily quantified in all spice paprika produce samples except, of course, for the untreated control. Based on our measurements, the level of pesticide residues correlated well with the number of treatments and the applied dosages, therefore the higher doses of pesticide treatments resulted in a higher level of pesticide residues and lower levels of tocopherols and carotenoids. Highest residue level of chlorpyrifos, penconazole, and cypermethrin were found to be 1.75, 0.763, and 0.339 µg/g, respectively. Levels of tocopherols (up to 13%), carotenoids (up to 16%), red and yellow pigments (up to 5%) all decreased due to the treatments. During food processing, the stability of the pesticide residues was observed.



Figure 1. The semi-field modelling spice paprika cultivation plot in Budapest, Hungary. Extract samples are shown in the insert demonstrating high variability in the colour intensity of the extracts (1: standard solution, 2-6: samples of paprika varieties ordered by colour depth of the extracts (Mörtl et al., 2001) - all varieties subjected to the same extraction procedure) (bottom right)

The results of the experiment revealed that increasing use of pesticides (both in terms of increasing dosages and multiple usage times) obviously leads to increased pesticide residue levels in the produce. Residue levels detected correlated well with pesticide treatments at increasing dosages applied. This was particularly observed for chlorpyrifos that was found even in samples treated with low dose. This justifies the ban of this active ingredient in the EU on all commodities including red pepper. Contamination occurred at high doses of active ingredients penconazole and cypermethrin, as well.

In addition to the obvious pesticide residue problem at high dosages, the experiments also clearly indicated that chemical stress by exposure to the pesticides triggered a reduction in the production of beneficial bioactive substances in the paprika produce e.g., high dosages of pesticide treatment resulted in lowered levels of endogenous tocopherols (up to 13%) and carotenoids (up to 16%) (Figure 2).

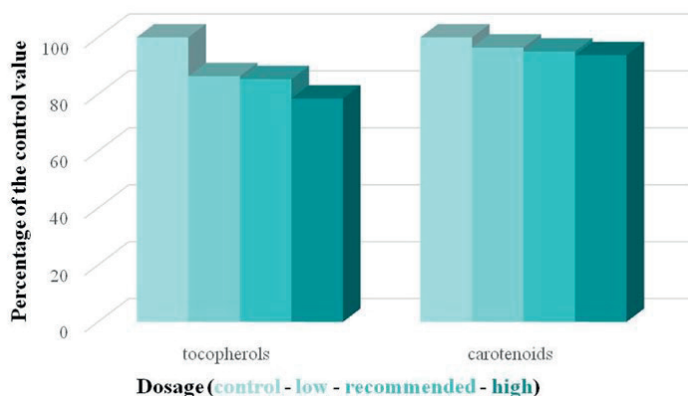


Figure 2. Endogenous levels of bioactive compounds in spice paprika at various pesticide treatment dosages

### POSSIBLE EFFECTS BY PESTICIDE ADDITIVE SUBSTANCES - MAINLY THE FORMULATING AGENTS

Various pesticide formulations contain different additives (e.g., formulating agents) besides their active ingredients. The primary purpose of formulating agents used in PPPs is to enhance the efficacy and bioavailability of the formulation by increasing the water solubility and uptake, in addition to the enhancement of the adsorption capacity, environmental stability, and biological activity of the active ingredients resulting in more efficient penetration of pesticide compounds and more effective plant protection treatment (Broze, 1999; Castro et al., 2014). Previously different additives (e.g., formulating agents) in PPPs have been classified as inactive components in terms of the main biological activity of the formulations. Although, in recent years, the high individual toxicity of formulating agents or the increased combined toxic effects of the active ingredients and additives were demonstrated. In the case of glyphosate-based herbicides, the outstanding individual toxicity of the formulating agent (a mixture of polyethoxylated tallow amines; POEA) and high combined toxicity with the active ingredient was proved (Tsui & Chu, 2003; Mesnage et al., 2014), therefore the use of POEA as formulating agent in the glyphosate-based formulation has been banned (European Commission, 2016). Based on the scientific evidence, the possible adverse effects of the formulating agents in pesticide formulations and tank mixtures cannot be

excluded in terms of the food safety of agricultural products and the bioactive component content of crops.

### CONCLUSIONS

Due to improper use of pesticides in paprika cultivation, not correct conditions of storage and harvest, furthermore, the illegal use of chemical PPPs in commodities, residues of pesticide compounds may appear in agricultural food products, thus various physical, chemical, or biological agents may have potentially harmful effects on food safety of spice paprika. To support the promotion of appropriate food safety in the EU, an integrated concept “from farm to fork” is elaborated and applied, with risk assessment based on scientific facts and reports.

In 2019 the European Commission presented the European Green Deal and adopted a set of proposals to make Europe the first climate-neutral continent by 2050 with the improvement of the economy, human health, and life quality (European Commission, 2019; European Commission, 2022a). One of the aims of the European Green Deal is a 50% reduction in the use and risk of chemical pesticides, at least 50% and 20% reduction in nutrient losses and fertilizer use, moreover a 25% increase in organic agriculture by 2030.

Organic farming is an environmentally friendly way of agricultural practice that needs to be further developed. From 2009 the demand for organic farming in spice and herb cultivations increased in the EU to avoid the exposure of consumers to pesticide residues in spices and

herbs, although the consumed quantities vary by various products and regions (CBI 2009). Moreover, the beneficial effects of organic farming on antioxidant activity, total carotenoids, the content of minerals, vitamin C, flavonoids, and color intensity of paprika were proved by several studies (Daood & Biacs, 2005; Hallmann et al., 2007; Pérez-Lopez et al., 2007; Hallmann & Rembalkowska, 2012). Emphasis is recently put on the use of pepper traditional varieties/landraces, giving their great genetic diversity, on the application of different strategies for plant protection (Sánchez-Sánchez et al, 2022), as biologic control, plants extracts, allelopathy, intercropping, (Rodino et al., 2017), use of N-fixing and K and P solubilizing microbial biostimulants bacteria (Sánchez-Sánchez et al, 2022), plant growth -promoting bacteria and arbuscular mycorrhizal fungi (Jimenez-Perez et al., 2022). The present collaboration within the Erasmus+ Hort4EUGreen project supports the dissemination of pesticide-free and organic farming knowledge and nutritional quality of horticultural products knowledge for students and whoever other interested actors.

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