Chemical Control of Olive Fungal Diseases: Strategies and Risks

Kemijska kontrola gljivičnih bolesti maslina: mogućnosti i rizici

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CHEMICAL CONTROL OF OLIVE FUNGAL DISEASES: STRATEGIES AND RISKS

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Scientific review
Pregledni znanstveni članak

SUMMARY

An increase in olive production requires a greater use of plant protection agents. Despite the advancements in alternative methods of disease control, chemical substances are anticipated to remain a primary measure due to their cost and effectiveness. Currently, the Phytosanitary Information System Database of the Ministry of Agriculture lists 17 registered fungicides specifically approved for controlling the pathogenic fungi affecting olive trees in Croatia. Fungicides containing copper and sulfur are most commonly used, along with the formulations based on tebuconazole, prochloraz, and trifloxystrobin. However, the chemical compounds found in fungicides can often impact the environment. An improper application of fungicides can result in adverse effects in the food chain, leading to the accumulation of residues from the plant-protection agents and posing risks to human and animal health. Therefore, in agriculture, phytomedicine is appropriately regulated by the laws comprehensively. This study delves into specific chemical agents for controlling fungal diseases in olives and assesses the associated hazards.

Keywords: fungicide, kresoxim-methyl, Olea europaea L., tebuconazole, trifloxystrobin

INTRODUCTION

Olives are an integral part of Mediterranean culture. In Croatia, with a rich tradition of olive cultivation, they hold a profound place in the agricultural landscape. The high-quality extra virgin olive oils, obtained from the indigenous olive varieties, represent a distinctive product in both the domestic and international markets. It is widely believed that this culture dates back more than 45,000 years (Žužić, 2008). The intensified cultivation of olive trees has significantly increased a need for the application of plant-protection agents. The emergence of resistance to phytopathogenic organisms poses a significant challenge for the producers as well. A prolonged exposure, or an incorrect utilization of chemical agents, may have detrimental effects on the health of agricultural workers and consumers.

Although the number of studies on the application of biological methods for plant-disease control is increasing, chemical control remains the most widely used form of crop protection. The reasons for this are the effectiveness of chemical agents, their availability on the market, lower cost, and the like. In the agricultural sector, phytomedicine is subjected to a comprehensive regulatory framework, evident from various laws and regulations (Havranek et al., 2014). An improper and excessive use of chemical plant-protection products can lead to undesirable consequences. The analyses indicate that the amount of residues in food is increasing (Ministry of Agriculture, 2020), thus highlighting a need for the judicious use of plant-protection agents.

This study aspires to elucidate specific chemical agents considered suitable for the control of fungal diseases in olive trees in Croatia. Additionally, it provides a comprehensive assessment of the intrinsic hazards associated with their application. The exploration of these chemical agents and the evaluation of their potential risks are crucial steps in understanding the complexities involved in utilizing the plant-protection substances in olive cultivation.

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FUNGAL DISEASES OF OLIVES IN CROATIA

Nowadays, the diseases described as the most important olive diseases worldwide are either poorly known in Croatia or, until now, have not inflicted significant damage due to their occurrence (Godena et al., 2018). However, Peacock spot disease caused by the *Venturia oleaginea* (Castagne) Rossman & Crous (syn. *Spilocaea oleaginea*), Verticillium wilt caused by the *Verticillium dahliae* Klebahn, anthracnose caused by the *Colletotrichum* spp., and fungal canker caused by the species from the *Botryosphaeriaceae* family are reported as the common olive fungal diseases in Croatia (Cvjetković, 2010; Miličević et al., 2012; Kaliterna et al., 2016; Godena et al., 2018; Godena et al., 2022; Petrović et al., 2023a). Milčević et al. (2012) reported the occurrence of fruit anthracnose in Croatia to a lesser extent, although it can still have a significant presence in some years. Buljubašić et al. (2012) and Cvjetković and Vončina (2012) identified olive anthracnose as the most frequent disease in Croatia, while *V. dahliae* was the most common causal agent of olive diseases in a research counducted by Godena et al. (2018).

Botryosphaeria dieback is a disease that causes branch and twig dieback, necrotic lesion-like appearance, rot and fruit drop, leaf drying and defoliation, and the like. The species from this genus can cause significant and economically important diseases (Kaliterna and Milčević, 2012). In Croatia, the species *Botryosphaeria dothidea* (Moug.) Ces. & De Not. (Cvjetković, 2010), *Diplodia seriata* De Not. (Kaliterna et al., 2012; Godena et al., 2018; Ivić et al., 2023), and *Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips (Ivić et al., 2023) have been identified. Locally and globbaly, the most well-known and widespread disease is fruit rot, also known as the Dalmatian disease or patula, caused by the species *B. dothidea* (Kaliterna and Milčević, 2012). The disease symptoms can easily be confused with olive anthracnose caused by the *Colletotrichum* spp., whereas the disease may be mistaken for the Verticillium wilt due to a symptom decline (Kaliterna and Milčević, 2012). The occurrence of these fungi is particularly increased subsequent to an olive damage caused by the low temperatures and pruning.

The *Colletotrichum* spp. have been recognized as causing fruit rot or anthracnose in olives. One of the most common pathogens is the *C. gloeosporioides* (Penz.) Penz. & Sacc. The older name for this fungus is *Gloeosporium olivarum* J. V. Almendia, and it can be found in literature under that name (Cvjetković, 2010). The disease is known in the entire Mediterranean olive-growing region. The oil obtained from the infected fruits is reddish in color, cloudy, and of poor organoleptic properties (Cvjetković, 2010). Some symptoms of the disease include the appearance of dark spots on fruits, rot and fruit drop, the appearance of yellow chlorosis on the leaves, and defoliation.

The Verticillium wilt is described as one of the most aggressive olive diseases (Jiménez-Díaz et al., 2012). The damage caused by the *V. dahliae* result in the reduced yields in older trees, while complete wilting can occur in the younger trees (Godena et al., 2018). The typical symptoms include dieback, with the branches exhibiting vascular browning in the cross-section and the attached, light brown and downwardly curled leaves (Kaliterna et al., 2016). The appearance of this disease can limit the tree growth and productivity (Godena et al., 2022).

The peacock spot disease, described in Croatia as early as in 1901 (Vrsalović, 1901), exhibits the characteristic symptoms, such as the appearance of green to bluish spots surrounded by several concentric rings of different colors. As the disease progresses, the spots become dark brown (Cvjetković and Vončina, 2012). In the last stage, the spots become whitish due to the detachment of the cuticle from the lower side of the epidermis (Buljubašić et al., 2012). The symptoms can appear on the fruit and the fruit stalk too (Cvjetković and Vončina, 2012). The signs of the disease can be present throughout the year, with the intensity varying by season, as climatic factors are crucial in the symptom appearance. Additionally, the occurrence of the disease depends on a variety's susceptibility, and it more commonly occurs on the trees with dense canopies, on the locations where a high relative humidity persists (Cvjetković and Vončina, 2012). Since this disease causes significant economic losses, it is alarming that infection rates of up to 100% have been recorded on some locations in Croatia, which exceeds the infection tolerance threshold of this fungus, set at 10% (Buljubašić et al., 2012). The disease is often underestimated due to the difficulty to reveal the early leaf symptoms and the pathogen-induced phyllotopsis, which creates an illusion of healthy and restored plants (Bounaurio et al., 2023).

The less common causal agents of fungal diseases of olives in Croatia include the species *Armillaria mellea* (Vahl) P. Kumm. (Godena et al., 2018), *Biscogniauxia* spp. (Petrović et al., 2024), *Comoclathris incompta* (Sacc. & Martelli) Ariyaw. & K.D. Hyde (syn. *Phoma incompta* Sacc. & Mart.) (Ivić et al., 2010), *Cytospora pruinosa* Défago (Petrović et al., 2023b), *Diaporthe* sp. (Ivić et al., 2023), *Nigrospora* spp. (Petrović et al., 2023c), *Phaeoacremonium iranianum* L. Mostert, Gräfenhan, W. Gams & Crous (Petrović et al., 2022), *Pleurostoma richardsiae* (Nannfeldt) Réblová & Jaklitsch (Ivić et al., 2018), *Pseudocercospora cladosporioides* (Sacc.) U. Braun (Milčević et al., 2012), and *Sordaria fimicola* (Roberge ex Desm.) Ces. & De Not (Petrović et al., 2024).

A control of the pathogenic fungi, such as those from the *Botryosphaeriaceae* family, is difficult and requires meticulous attention (Kaliterna and Milčević, 2012).

CHEMICAL CONTROL OF FUNGAL OLIVE DISEASES

To control fungal diseases, the prevailing practice involves preventive measures (e.g., pruning, the removal of debris, etc.) and the application of fungicides and formulations containing copper and sulfur. Nevertheless, it is imperative to note that those products exhibit a limited efficacy against the latent infections and vegetative mycelium residing both on and beneath the bark of the infected trees (Moral et al., 2008). The fungicidal efficacy of copper emanates from the presence of copper (II) ions, which disrupt the enzymatic reactions within the fungal spores, thereby inducing an inhibition of their germination. Sulfuric compounds within the cellular structures disrupt the redox reactions by competitively engaging with oxygen as a hydrogen acceptor. Currently, the Phytosanitary Information System Database of the Ministry of Agriculture (FIS, 2024) lists 17 registered fungicides specifically approved for use in the control of pathogenic fungi impacting olive trees in Croatia (Table 1).

The table reveals that the majority of fungicides are formulated for the control of the *V. oleaginea*, which contributes to reduced yields and economic losses. The chemical protective measures against a substantial number of phytopathogenic fungi are largely absent, with the preventive strategies being a predominant recourse. Tebuconazole and prochloraz emerge as the most efficacious active ingredients in fungicides for the management of the *Diplodia mutila* (Fr.) Fr., *D. seriata*, *Neofusicoccum australe* (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips, and the *N. parvum* (Bester and Fourie, 2005; Savocchia et al., 2005; Amponsah et al., 2012). Additionally, the formulations based on carbendazim, thyophanate-methyl, and pyraclostrobin, alongside tebuconazole itself (Wang et al., 2022) are indicated as effective against the *B. dothidea.* The *Colletotrichum* spp. can be managed by the application of ciprodinil, difenoconazole, carbendazim, and tebuconazole in the concentrations ranging from 0.12 to 2.69 g/ml. The application of these substances resulted in a 50% inhibition of conidia germination in comparison to the controls (Nawaz et al., 2023). A pre-harvest fungicide application significantly reduces the anthracnose infection on olive fruits by 70 to 90%, with a post-harvest application reducing the disease severity and spread by 75 to 95% (Nawaz et al., 2023). The chemical protective measures of notable significance for the management of the *V. dahliae* are currently unknown. In the literature, the data are provided regarding a potential application of prochloraz, whose antifungal efficacy was examined in the *V. dahliae* isolates from the cotton plants, revealing a pronounced control of the disease at higher concentrations of the prochloraz-manganese combination (Kurt et al., 2003). Bubici et al. (2019) investigated a possibility of applying the tebuconazole on the artichokes and potatoes *in vitro*, in a greenhouse, and in the field trials. The *in vitro* assays affirmed the tebuconazole as highly effective against the *V. dahliae*, while the mixed results were obtained in the greenhouse and field experiments. A greenhouse application reduced the *V. dahliae* incidence on the artichokes but had no impact on the symptom intensity and necrosis of conductive bundles. Conversely, the tebuconazole completely suppressed the disease and stimulated plant growth when applied to the potted potatoes. In the field trial with the heightened *V. dahliae* incidence, no significant disease control was observed subsequent to the tebuconazole application. In the studies on peppers, benomyl was the most effective fungicide in controlling the *V. dahliae*, amounting to 88.2–94.6% (Rekanović et al., 2007). Regrettably, the literature contains scant data on the effective chemical protection methods, with an absence of information regarding their applicability on the olive trees against the *V. dahliae*. Recent investigations have primarily focused on biological control methods. Therefore, it would be necessary to conduct fungicide testing on the pathogens isolated from the olives, as well as on the olive trees themselves. A testing on the olive trees allows for the assessment of real conditions under which the fungicides are applied and their ability to cope with environmental variations. This approach also enables the identification of potential side effects or unwanted consequences of fungicide application on the olive trees. A testing on the trees contributes to the comprehension of how the fungicides react in natural conditions, including the aspects such as the effectiveness of disease control, potential phytotoxic effects, and their impact on the overall health and development of olive trees.

Table 1. A list of the fungicides approved for the use in olive cultivation in Croatia, including their registration owner, type, active substances and quantity, and the targeted phytopathogenic organisms (FIS, 2024).

Tablica 1. Popis odobrenih fungicida za upotrebu u uzgoju maslina u Hrvatskoj, uključujući vlasnika registracije, vrstu i oblik, aktivnu tvar i štetni organizam na koji djeluje (FIS, 2024.).

Fungicide / *Fungicid*	Registration owner / *Vlasnik registracije*	Type / Form *Vrsta / Oblik*	Active substance and quantity / *Aktivna tvar i količina*	Targeted phytopathogenic organism / Štetni *organizam na koji djeluje*
COSAVET DF	Sulphur Mills Limited	Contact preventive fungicide/ Water-dispersible granules *Kontaktno preventivni fungicid / vododispergirajuće granule*	Sulphur *Sumpor* 800 g/kg	*Leveillula* spp.
NATIVO 75 WG	BAYER AG	Combined fungicide/water-dispersible granules *Kombinirani fungicid / vododispergirajuće granule*	Trifloxystrobin *Trifloksistrobin* 250 g/kg Tebuconazole *Tebukonazol* 500 g/kg	*Venturia oleaginea* (=*Spilocea oleaginea*)
NEORAM WG	Gowan Crop Protection Limited	Contact preventive fungicide/ Water-dispersible granules *Kontaktno preventivni fungicid / vododispergirajuće granule*	Copper oxychloride *Bakarni oksiklorid* 375 g/kg	*V. oleaginea*
NORDOX 75 WG	Nordox AS	Contact preventive fungicide/ water-dispersible granules *Kontaktno preventivni fungicid / vododispergirajuće granule*	Copper oxychloride *Bakarni oksiklorid* 750 g/kg	*V. oleaginea*
CUPRA	LAINCO S.A.	Fungicide/suspension concentrate *Fungicid / koncentrat za suspenziju*	Copper oxychloride *Bakarni oksiklorid* 520 g/l	*V. oleaginea*
COPPER KEY	INDUSTRIAL QUIMICA KEY S.A.	Fungicide / wettable powder *Fugicid / močivo prašivo*	Copper oxychloride *Bakarni oksiklorid* 577.2 g/l	*V. oleaginea*
CODIMUR 50	Exclusivas Sarabia S.A.	Fungicide / wettable powder *Fugicid / močivo prašivo*	Copper oxychloride *Bakarni oksiklorid* 877.2 g/l	*V. oleaginea*
COPPER LAINCO	LAINCO S.A.	Fungicide / eettable powder *Fugicid / močivo prašivo*	Copper oxychloride *Bakarni oksiklorid* 877.2 g/l	*V. oleaginea*
CUPRABLAU Z 35 WG	CINKARNA metalurško-kemična industrija Celje d.d.	Fungicide / water-dispersible granules *Fungicid / vododispergirajuće granule*	Copper oxychloride *Bakarni oksiklorid* 350 g/kg	*V. oleaginea, Capnodium* sp.
AIRONE SC	Gowan Crop Protection Limited	Fungicide / suspension concentrate *Fungicid / koncentrat za suspenziju*	Copper oxychloride *Bakarni oksiklorid* 136 g/l	*V. oleaginea, Pseudocercospora cladosporioides*
SERENADE ASO	BAYER AG	Biological fungicide / suspension concentrate *Biološki fungicid / Koncentrat za suspenziju*	*Bacillus amyloliquefaciens*	*V. oleaginea, Colletotrichum* spp.
SUGOBY	LAINCO S.A.	Fungicide / water-dispersible granules *Fungicid / vododispergirajuće granule*	Kresoxim-methyl *Krezoksim-metil* 500 g/kg	*V. oleaginea*
QUIMERA	INDUSTRIAS AFRASA, S.A.	Fungicide / water-dispersible granules *Fungicid / vododispergirajuće granule*	Kresoxim-methyl *Krezoksim-metil* 500 g/kg	*V. oleaginea*
AZUMO WG	Azufrera y Fertilizantes Pallares, S.A.U.	Fungicide / water-dispersible granules *Fungicid / vododispergirajuće granule*	Sulphur *Sumpor* 800 g/kg	*Capnodium* sp.
VISUL WG	Azufrera y Fertilizantes Pallares, S.A.U.	Fungicide / water-dispersible granules *Fungicid / vododispergirajuće granule*	Sulphur *Sumpor* 800 g/kg	*Capnodium* sp.
CODIMUR SC	Exclusivas Sarabia S.A.	Fungicide / suspension concentrate *Fungicid / koncentrat za suspenziju*	Copper oxychloride *Bakarni oksiklorid* 520 g/l	*V. oleaginea*
COPPER KEY FLOW	Industrial Quimica Key S.A.	Fungicide / suspension concentrate *Fungicid / koncentrat za suspenziju*	Copper oxychloride *Bakarni oksiklorid* 520 g/l	*V. oleaginea*

In the context of phytosanitary chemical measure employment, a potential exists for the persistence of active residues withins food chain, which can lead to the undesirable consequences on both the human and animal health. In the food chain, the accumulation of residues of plant protection agents (PPAs) used in the protection of plants in the process of livestock feed production may occur (Havranek et al., 2014). The PPA residues in the food are harmful to human health due to their fat solubility, accumulation in human adipose tissue, and their prolonged action due to a high biochemical and chemical stability, or persistence (Havranek et al., 2014). The endocrine disruptors typically accumulate in the adipose tissue, exhibit a high resistance to the metabolic processes and degradation, and possess a notable capacity for bioconcentration within the food chain. They can act chronically even in the very small quantities, and the effect of substances depends on the duration of exposure to the humans and animals. The endocrine disruptors can be bound to the hormone receptors, mimicking or antagonizing the physiological functions of hormones. They can stimulate or inhibit the enzymes responsible for the synthesis or for the release of hormones, thereby increasing or decreasing the action of hormones (Bagi and Bodnar, 2012). Some substances can be released during breastfeeding in breast milk (Springer and Springer, 2008), bind to the plasma proteins and induce methemoglobinemia, accumulate in liver, and affect the immune activities of an organism (Čižek and Vajdon, 2007; Springer and Springer, 2008). Breast milk may contain a significantly higher concentration of harmful substances than other food, in some cases 10 to 20 times higher than the cow's milk (Kožul et al., 2010). Some active substances have adverse effects such as carcinogenicity, mutagenicity, or teratogenicity.

Among the fungicides listed in Table 1 and used for controlling fungal diseases in olives, several of them exhibit the pronounced toxic effects on humans and animals. The *Cosavet* fungicide is noted for skin irritation, while *Cuprablau Z 35 WG* causes severe eye irritation. The fungicides *Azumo WG* and the *Visul WG* can also cause skin irritation (AFEPASA, 2018, 2020). The concerns about possible carcinogenic effects have been raised regarding the fungicides *Sugoby* and *Quimera*. Additionally, the fungicides like *Airone SC*, *Codimur 50*, *Copper Key*, *Copper Lainco*, *Cupra*, *Cuprablau Z 35 WG*, *Neoram WG*, *Nordox 75WG*, *Quimera*, and *Sugoby* pose a notable threat to aquatic environments (FIS, 2024).

Elemental sulfur contained in the aforementioned sulfur-based fungicides (Table 1) is of a relative low toxicity for the humans and animals in its pure form. However, its toxicity can vary depending on the form, dose, mode, and the duration of exposure, as well as depending on the individual organism characteristics. For example, inhaling the large amounts of sulfur powder can cause respiratory tract irritation, direct contact with the skin can cause irritation, swallowing large amounts can lead to gastrointestinal disturbances, and the like. Once sulfur is released into the environment, it is rapidly oxidized by the bacteria, other microorganisms, or spontaneously by the presence of oxygen to transform into the organic sulfur compounds (AFEPASA, 2018). Sulfur is generally considered safe for human exposure, as it is naturally present and abundant in food. Toxicological studies have proven that sulfur has a low acute oral, dermal, and inhalation toxicity. The acute and short-term risk to the insectivorous birds and mammals was assessed as low for the use in cereals and vineyards, but there is uncertainty regarding a long-term risk to birds and mammals. The risk to the aquatic organisms was generally considered low, as sulfur's solubility in water is very high (EFSA et al., 2023).

Copper oxychloride contained in the aforementioned copper-based fungicides (Table 1) is harmful if inhaled or ingested. The targeted organs are liver and kidneys. A concern has been expressed regarding copper inhalation, as the lung lesions observed in an operator exposure were reproducible in the guinea pigs. Genotoxicity was not a concern with oral administration; however, there was insufficient evidence to exclude the genotoxic potential of copper subsequent to a non-oral exposure. No carcinogenic potential of copper was determined either in rats or in humans. Copper did not induce adverse effects on fertility, reproductive parameters, or development, and neurotoxicity was not attributed to the copper ingestion. A risk assessment suggests a low acute and short-term risk for birds. However, copper is highly toxic to the aquatic organisms (EFSA, 2008b).

Despite the aforementioned facts, according to the Regulation on Organic Production in Plant Cultivation and Production of plant products (*Official Gazette* 91/2001), the preparations based on copper oxychloride and sulfur can be used in organic agriculture in Croatia.

The fungicide *Nativo 75 WG* is suspected to have potential adverse effects on fertility or a possibility inflict harm to an unborn child and is highly toxic to the aquatic organisms, with the long-lasting effects. It is one of the most commonly used fungicides in practice for the control of fungal diseases in olives. This fungicide contains the active substances tebuconazole and trifloxystrobin. Tebuconazole is effective against a wide range of fungi, including fungal species from the *Botryosphaeriaceae* family. Tebuconazole is the approved name for the (RS)-1-p-chlorophenyl-4,4-dimethyl-3-(1H-1,2,4-triazol-1-ylmethyl)pentan-3-ol and was initially used in 1989 for the treatment of cereals. It is a triazole fungicide that functions by impeding the sterol biosynthesis in fungi. Its toxicity was incipiently documented in 1994 (FAO, 2023). Tebuconazole is rapidly and completely absorbed within 48 hours and exhibits a rapid and extensive elimination, being excreted via feces by 65% to 80% and via urine by 16% to 35% (EFSA, 2008a). Georgopapadakou (1998) states that tebuconazole manifests its fungicidal activity by inhibiting the fungal cytochromes P450 (CYP) 51—that is, lanosterol-14α-demethylase. As a side effect, tebuconazole can inhibit the mammalian CYP enzymes, including those essential for the synthesis of steroid hormones (e.g., aromatase, CYP19A1) or for the metabolism of vitamin A (Zarn et al., 2003; Vinggaard et al., 2006; Robinson et al., 2012). In the short-term toxicity tests conducted on the rats and canines, tebuconazole induced the adrenal gland hypertrophy and was classified as a cause of malformations in several species (EFSA, 2014a). Liver is the most sensitive targeted organ for triazoles, attributed to their interaction with the nuclear liver receptors, such as the constitutive androstane receptor (CAR), leading to an increased liver weight and centrilobular hypertrophy in rats and mice (Goetz and Dix, 2009; EFSA, 2014a; Schmidt et al., 2016). Besides liver, it causes damage to the adrenal glands (EFSA, 2008a; FAO, 2023). Tebuconazole's impact on the aquatic organisms has been documented. The exposure of the zebrafish larvae (*Danio rerio*) to tebuconazole at the maximum non-lethal concentration from 72 to 120 hours post-fertilization resulted in notable alterations in liver size and yolk retention in the zebrafish larvae. A discernible decline in fluorescence intensity was observed in the Tg(Apo14:GFP) zebrafish larvae, signifying a liver degeneration subsequent to the tebuconazole treatment. These results suggest that tebuconazole exposure poses an ecological threat to the fish and vertebrates (Li et al., 2020). In a study presented by the California Environmental Protection Agency, Pesticide Regulation Branch, in 2006, it was noted that tebuconazole may impact fertility. Fetal laboratory rats treated with tebuconazole manifested a reduced body weight, with the cases in which the rats lacked their tails or had deformed faces, and, in some instances, a decrease in litter size was observed. The teratogenic effects were also noted in rabbit testing (FAO, 2023). Additionally, dermal and oral toxic effects were recorded in rat testing (EFSA, 2008a.). It has recently been discovered that tebuconazole may have a cardiotoxic effect, causing an increased myocardial fibrosis in the male Wistar rats at the doses of approximately 30 mg/kg (Othmène et al., 2020). At higher concentrations, tebuconazole exhibited carcinogenic effects in mice and rats (FAO, 2023). In a 2019 study (Knebel et al., 2019.) investigating the ability of tebuconazole to activate gene expression via aryl hydrocarbon receptor (AHR), the results demonstrated a statistically significant induction of the AHR target genes—that is, of the CYP1A and CYP1A2, in human liver cells under *in vivo* conditions. Habenschus et al. (2021.) demonstrated the impact of tebuconazole on the human CYP450 enzyme, the most pivotal enzyme involved in drug metabolism.

Trifloxystrobin is an active substance classified within the strobilurin group. Its mode of action involves the inhibition of fungal cell respiration, disrupting the mitochondrial function and interfering with the energy-production process, ultimately leading to the fungal cell death. Trifloxystrobin is generally considered to have a low level of toxicity. However, some studies suggest potential adverse effects. Jang et al. (2016) investigated the impact of trifloxystrobin on the skin at the cellular level utilizing the HaCaT—that is, the human keratinocyte cells. The cells were exposed to trifloxystrobin for 48 hours, revealing detrimental effects on the mitochondria, indicating trifloxystrobin's potential role as a contributor to the development of skin disorders.

Kresoxim-methyl, which is also a strobilurin-derived active compound, acts by inhibiting the germination of fungal spores and the growth of mycelium. While its harmful impact on the aquatic organisms is well-documented, the precise mechanisms underlying its toxicity remain inadequately explored. In a study conducted on the zebrafish species, negative effects were observed on the development of liver, ovaries, and intestines, influencing digestion and the absorption of vitamins, retinol metabolism, and more. It was noted that it could affect the energy supply for normal development of liver and oocytes (Fang et al., 2022).

The fungicide *Serenade ASO* is the only biological fungicide among the listed fungicides in Table 1. It contains the antagonistic microorganism *Bacillus amyloliquefaciens* (formerly *B. subtilis*), strain QST 713. Also, it contains the naturally occurring bacteria that are not genetically modified. The *Serenade ASO* prevents the plant diseases while initially creating a zone of inhibition on the leaf and preventing the attachment and penetration of pathogens. The biological compounds produced by the *B. amyloliquefaciens* strain QST 713 act to destroy the germ tubes and mycelia of the pathogenic fungi by puncturing their cell membranes (Bayer, 2021). Sabo et al. (2020) investigated the impact of the fungicide *Serenade ASO* on the winter honeybees. Despite no direct lethal effect of the commercial plant-protection product containing the *B. amyloliquefaciens*, the strain QST 713 was observed in the adult bees following a chronic exposure; thus, the decrease in some immunity parameters observed in the tested winter bees may potentially impair bee colony health and survival. In the area of ecotoxicology, a low risk was concluded for the birds, wild mammals, aquatic organisms (excluding plants), and non-target arthropods (EFSA, et al., 2021).

The maximum residue level (MRL) of tebuconazole in olives and olive oil is 0.05 mg/kg (EFSA, 2018.). Trifloxystrobin has a MRL of 0.2 mg/kg for table olives and 0.05 mg/kg for the olives designated for oil production (EFSA, 2014b). Kresoxim-methyl has a MRL of 0.3 mg/kg for both the table olives and those used in oil production (EFSA, 2014c). The MRL of copper in table olives and the olives for oil production is 20 mg/kg (EFSA et al., 2022). Regarding sulfur, it is unnecessary to consider the residues because a toxicity assessment for mammals concluded that sulfur is of low toxicity. Therefore, a consumer risk assessment is neither possible nor necessary (EFSA, 2023). However, a general default MRL of 0.01 mg/kg applies when a pesticide is not specifically mentioned (European Commission, 2024). The MRL, typically applied in the case of the chemical pesticides, is not available for the *B. amyloliquefaciens* strain QST 713. Considering the uncertainties associated with the occurrence of non-viable residues (metabolites) of the *B. amyloliquefaciens* strain QST 713, especially concerning their quantities on the edible crops at harvest, a consumer risk assessment cannot be concluded. The *B. amyloliquefaciens* strain QST 713 has a potential to produce several secondary metabolites such as the bacillaene, bacillibactin, bacilysin, difficidin, ericins, fengycins, iturins, macrolactin, and surfactins. A toxicological profile of these metabolites is not conclusively elucidated (EFSA et al., 2021).

Current methods for the analysis of tebuconazole, trifloxystrobin, and kresoxim-methyl in food involve a gas and liquid chromatography following sample extraction with the organic solvents. These methods offer efficiency, reproducibility, and accuracy, but they remain time-consuming and costly. Chromatography is notably one of the most common techniques for pesticide analysis in food and beverages. Additionally, techniques such as electrophoresis, infrared spectroscopy, and mass spectroscopy are applied. Long Truong et al. (2021) highlight a potential for rapid detection of tebuconazole based on the aptasensors and silver nanoparticle aggregation. Monitoring the levels of pesticide residues in food and beverages is crucial for the maintenance of food safety. According to the latest data, or the Report on the Results of the National Program for Monitoring Pesticide Residues in Food for the year 2021, out of all collected samples of olive oil in Croatia (23), none of them were detected to have the pesticide residue levels above the MRL (FIS, 2021).

A large number of new PPas are introduced to the market each year. In some cases, it may take several years to observe their impact on human and animal health. The residues of pesticides such as the polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT) have been documented even in Antarctica. The PPAs can infiltrate into the human and animal systems via multiple pathways, for instance, by ingestion via food and beverages, by absorption through the digestive organs, by dermal contact, and by inhalation. The immediate consequences may be manifested as dermatological issues or as a gastrointestinal discomfort, while an internal organ damage might become apparent over time. The workers handling pesticides directly, be it during the application, production, or transportation, are exposed to potential health risks. It is imperative to adopt protective measures, such as wearing specialized clothing during pesticide handling, to minimize the associated risks. Furthermore, the preparation of pesticides should strictly adhere to appropriate formulations.

Despite numerous drawbacks and potential hazards associated with their use, the PPAs continue to play a pivotal role in agriculture. They are cost-effective, provide adequate plant protection when applied correctly, and facilitate food production significantly.

CONCLUSION

Olive diseases pose a significant threat to agricultural yield and can result in substantial economic losses. The intensification of olive production further amplifies a necessity for robust plant protection measures, with chemical pesticides remaining a predominant choice of disease control. Notably, a majority of plant-protection products available on the Croatian market are specifically formulated to combat the causal agent of peacock's eye disease. The aforementioned issue poses a challenge to producers, given the rising occurrence of fungal diseases in olive cultivation. Despite their efficacy, the overreliance on chemical pesticides has led to the emergence of resistance in pathogenic organisms, presenting a formidable challenge in the maintenance of effective disease-management strategies. Therefore, it is necessary to increase the number of studies that will involve the investigation of effectiveness when applying specific fungicides to the phytopathogenic fungi and the studies concerning the impact of their application on olive trees. Furthermore, a pervasive use of pesticides contributes to their accumulation in the environment and the food chain, thereby introducing potentially serious health risks for both human and animal populations. The impact of active substances and pesticides on the environment, as well as on the health of living organisms, remains an area in which comprehensive research is warranted. A need for further testing to elucidate the full extent of their effects is crucial in understanding the potential long-term consequences. Effective monitoring and regulation of pesticide use are imperative to mitigate their adverse effects on the ecosystems. Appropriate dosing, a judicious selection of pesticides based on specific needs, and a strict implementation of protective measures during application are essential practices to minimize the negative consequences associated with the pesticide use. Addressing these challenges often requires the adoption of an integrated approach to agriculture, combining diverse plant-protection methods to implement sustainable and efficient agricultural practices.

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KEMIJSKA KONTROLA GLJIVIČNIH BOLESTI MASLINA: MOGUĆNOSTI I RIZICI

SAŽETAK

Povećanje proizvodnje maslina zahtijeva intenzivniju primjenu sredstava za zaštitu bilja. Unatoč napretku alternativnih metoda kontrole bolesti, kemijski fungicidi su i dalje ključni zbog svoje ekonomske isplativosti i učinkovitosti. Trenutačno, Fitosanitarni informacijski sustav Ministarstva poljoprivrede navodi 17 registriranih fungicida posebno odobrenih za suzbijanje patogenih gljiva na maslini u Hrvatskoj. Najčešće se koriste fungicidi na bazi bakra i sumpora, kao i formulacije na bazi tebukonazola, prokloraza i trifloksistrobina. Ipak, kemijski spojevi prisutni u fungicidima često mogu imati negativan utjecaj na okoliš. Nepravilna primjena fungicida može rezultirati nepoželjnim učincima u prehrambenome lancu, dovodeći do nakupljanja ostataka sredstava za zaštitu bilja i predstavljajući time rizik za zdravlje ljudi i životinja. Stoga je zaštita bilja u poljoprivredi adekvatno regulirana brojnim zakonima. Ovo istraživanje analizira specifične kemijske fungicide i tvari za kontrolu gljivičnih bolesti maslina u Hrvatskoj te raspravlja o potencijalnim opasnostima vezanima uz njihovu upotrebu.

Ključne riječi: fungicid, krezoksim-metil, Olea europaea L., tebukonazol, trifloksistrobin

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