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Fungicides in agriculture and their side effects on soil enzyme activities: a review

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Abstract

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A wide variety of fungicides are globally applied for effective elimination of fungal pathogens in agriculture. The constant increase in their production and use in the years rises a concern about the environmental effects that they can cause. More attention is paid on fungicides such as mancozeb, azoxystrobin, chlorotalonil, carbendazim, tebuconazole and captan due to their widespread application. The studies are focused on the analysis of parameters that could be rapid, sensitive and informative for the fungicides' impact on living organisms. Such parameter is the activity of soil microbial enzymes since their function is responsible for the soil health and fertility. Studies show that dehydrogenase, phosphatase and urease are the most commonly used enzymes due to their role in key metabolic processes, while invertase, β-glucosidase and cellulase were analyzed to a lesser extent. Most of the fungicides are reported to reduce the soil enzymes' activity while others manifest positive or controversial effects which is determined not only by the fungicide chemical composition but also by its dose, exposure time, and/or soil properties. The aim of the review is to summarize the results and outline the trends of fungicide impacts on soil enzymes that take part in the soil nutrient cycling.

Keywords: fungicides; soil microorganisms; enzyme activity

Introduction

The development of agriculture could be impossible without the application of pesticides since their main function is to control crop pests (Baćmaga et al., 2015) and hence provide the growing human population with food (Wang et al., 2020). Among pesticides, fungicides are the most used agrochemicals due to the susceptibility of crops to fungal diseases, being a major threat to food production (Gooding & Davies, 1997; Dardis & Walsh, 2000; Garthwaite et al., 2002).

In 2019 the global fungicide market size is amounted to USD 16.35 billion as it is predicted to increase at a compound annual growth rate of 4.3% until 2027. This is also caused since there are a lot of agrarian regions (India, Indonesia, South Korea, Thailand, Mexico, Central and South America) in the world that rely mainly on agriculture production (Grand View Research, 2020). In the European Union, fungicide sales (based on mass) account for more than 40% of the total pesticide sales with synthetic, organic fungicides accounting for approximately 60% of all sold antifungal compounds (Zubrod et al., 2019).

Concerning the mechanism of action, fungicides are classified as:

Inhibitors of ergosterol synthesis – block the synthesis of cell membrane and affect its integrity. Different fungicides can inhibit different stages of ergosterol synthesis. Some of the fungicides belonging to this group are: imidazole prochloraz and triazoles difenoconazole, myclobutanil, propiconazole, tebuconazole, triadimefan and tridemorph.

Inhibitors of mitochondrial electron transport (inhibitors of cellular respiration) – inhibitory effect can be on (1) succinate dehydrogenase activity, (2) cytochrome-bc1-complex (ubiquinol-cytochrome c-oxidoreductase) and (3) oxidative phosphorylation (ATP synthase). One of the main analyzed fungicides of this group are boscalid, azoxystrobin, trifloxystrobin, fluazinam, etc.

Others: fungicides with multi-site activity and fungicides-inhibitors of nucleic acid and protein syntheses. A number of compounds belonging to this fungicide group are synthesized in order not only to achieve a high efficiency against phytopathogens, but also to reduce the risk of resistance development. Their complex impact on cell processes makes it difficult for pathogens to develop multiple point mutations and create resistant strains. Some of the chemicals with multi-side activity are mancozeb, propineb, thiram, captan and chlorothalonil.

Despite the intensive use of fungicides and the associated potential for eco-toxicological risks on non-target organisms, the environmental effects of fungicides have received far less attention compared to insecticides and herbicides. For instance, Köhler & Triebskorn (2013) calculated that only 13% of the studies on pesticide effects between 1991 and 2013 are focused on fungicides, compared to 62% and 24% for insecticides and herbicides, respectively.

Application of fungicides, especially uncontrolled use may cause soil contamination with long-term negative effects on soil inhabitants and soil productivity (Baćmaga et al., 2015). Soil enzymes, synthesized mainly by soil microorganisms are important indicators of soil quality because of their immediate response to natural or anthropogenic changes in soil environments.

The present review attempts to summarize the information in the scientific literature on the effects of fungicides on soil enzymes, focusing on the fungicide dose and the enzyme responses in terms of their intensity, time and longevity of manifestation.

Fungicides of highest research interest

The review is based on the results of 45 authors reported in the period 2000 – 2020, who have studied the toxicity of a total of 28 fungicides. The first mentioned fact is that the number of tested active ingredients is very small compared to the large number of ingredients with antifungal activity (more than 580) that is marketed today (Wood, 2021). Some of the fungicides (azoxystrobin, carbendazim, chlorotalonil, mancozeb and tebuconazole) were analyzed in more than one study, and other fungicides (boscalid, fluazinam, iprobenfos, triazam, etc.) were found once in the literature.

Studies about the side effects of mancozeb, azoxystrobin, chlorotalonil, carbendazim, tebuconazole and captan comprise 50% of the total number of researches focused on fungicide stress on soil enzymes. The great interest to these agrochemicals is provoked by their widespread use due to their high efficiency on phytopathogens (mancozeb, chlorotalonil and azoxystrobin), high toxicity (chlorotalonil), and/or the large market share (mancozeb and azoxystrobin).

Market analysis shows that sold products containing mancozeb in 2007 worth approximately $ 740 million (Dow AgroSciences, 2008). About 85% of the sales are evenly distributed between Europe and the Asia-Pacific region, with only about 4% in North American markets. France, Italy, Spain and Portugal are among the countries in Europe with the most widespread use of mancozeb (Gullino et al., 2010).

The azoxystrobin market represents almost 46.51% of the total use of azoxystrobin for plant protection due to the growing demand for cereals. It is estimated at $ 460 million in 2017 and it will reach $ 1330 million by the end of 2025. Developing economies in the Asia-Pacific region (especially China and India) are expected to be the fastest growing azoxystrobin market in the next few years (QYResearch, 2018).

In the last few years, the development of chlorothalonil production has been unstable, with a growth rate ranging from 2.3% to 11.8%. In 2016, the actual production was about 35.3 thousand tons (Zhang, 2017). The global chlorothalonil market is estimated at $ 200 million in 2017, and is expected to reach $ 330 million by the end of 2023 (Report Hive Research, 2018), comprising growth rate of about 9%. With regard to the identified toxicity of chlorothalonil, the European Commission envisaged not renewing the approval of this fungicide after October 2019 and withdrawed existing products with chlorothalonil from the market in the first half of 2019 (Regulation 2019/677, 2019).

Effect of fungicides on soil enzymes

Data on the fungicide effects on 16 soil enzymes have been found in the literature. Some of the enzymes were tested by many authors using different fungicides, while others were less analyzed.

The most commonly used indicators of the fungicide effects are several soil enzymes – dehydrogenase, phosphatase and urease, and to a lesser extent – invertase, β-glucosidase and cellulase. In fact, 90%, 75% and 68% of the studied fungicides are tested for their side effects on phosphatase, dehydrogenase and urease, respectively. The focus on these enzymes is justified due to their key positions in metabolic pathways related to cell energy and nutrient supply.

Effects of fungicides on soil dehydrogenases

Soil dehydrogenases (EU 1.1.1.) are the main representatives of the oxidoreductases class (Gu et al., 2009). Dehydrogenases play an important role in biological oxidation of soil organic matter by transferring hydrogen from organic substrates to inorganic acceptors (Zhang et al., 2010). The total soil dehydrogenase activity depends on the activity of various specific dehydrogenases, which are a major part of the enzyme system of all living microorganisms, such as enzymes of respiratory metabolism, citrate cycle and nitrogen metabolism (Subhani et al., 2001). Thus, soil dehydrogenase activity serves as an indicator of microbiological redox systems and can be considered as a good and adequate measure for assessing microbial oxidative activity.

The highest interest in the effects of fungicides on soil dehydrogenase activity is shown for strobilurins (azoxystrobin), triazoles (myclobutanil, tebuconazole and triadimefon), benzimidazoles (benomyl and carbendazim), chloronitriles (chlorothalonil), carbamates (mancozeb and mixtures, and pirimicarb) and phenylamides (mefenoxam and metalaxyl).

In general, the reported effects of fungicides on the activity of dehydrogenase are negative, as they relate to the fungicide type and the applied dose (Baćmaga et al., 2015, 2020; Bello et al., 2012; Sun et al., 2020), and exposure time (Bending et al., 2007; Sopeña & Bending, 2013; Baćmaga et al., 2015; Guo et al., 2015; Wang et al., 2018). Stimulating effects are also observed, for example when benomyl was applied (Shukla, 2000; Chen et al., 2001).

The phenylamides mefenoxam and metalaxyl showed a strong toxicity on dehydrogenase at all applied concentrations (1 mg/kg – 1000 mg/kg). No recovery of enzyme activity was reported after mefenoxam (Monkiedje et al., 2002), while metalaxyl showed a weak stimulating effect after the 30-th/60-th day of soil amendment (Monkiedje et al., 2002; Sukul, 2006). For both fungicides, the effects on the enzyme were dose-dependent.

The significant effects of the strobilurin azoxystrobin on soil dehydrogenase, usually negative (Sopeña & Bending, 2013; Baćmaga et al., 2015; Guo et al., 2015; Álvarez-Martín et al., 2016; Wang et al., 2018), occured after prolonged exposure (30 – 60 days), but stimulating effects were also reported (Bending et al., 2007). Unlike other authors, Alvarez-Martin et al. (2016) did not report a significant effect of azoxystrobin on dehydrogenase activity in dose range of 0.2 mg/kg – 25.0 mg/kg and sampling occasion on 0 – 30 – 90 day.

The triazoles myclobutanil and triadimefon showed a weak stimulating effect on dehydrogenase activity at low concentrations (0.1 mg/kg – 0.68 mg/kg) and a short-term weak toxicity at higher concentrations (1 mg/kg and 10 mg/kg) (Deborah et al., 2013; Zhang et al., 2017).

Unlike all the fungicides considered so far, results obtained for tebuconazole are extremely contradictory, which provokes the idea that its action may depend on the environmental characteristics more than any other agrochemicals. For example, Bending et al. (2007) and Saha et al. (2016) found that doses below 5 mg/kg inhibit dehydrogenase activity. Bending et al. (2007) emphasized that after inhibition the enzyme not only recovered but was even stimulated compared to the control, and the authors related these fluctuations to soil characteristics, finding that the enzyme was weakly or completely unaffected in soils with high organic content. Wang et al. (2016) and Sun et al. (2020) observed enzyme inhibition at tebuconazole concentrations of 10 mg/kg (Wang et al., 2016; Sun et al., 2020) and 100 mg/kg (Wang et al., 2016), but not at 1 mg/kg (Wang et al., 2016). Baćmaga et al. (2020) reported reduction in enzyme activity under concentrations of 1.395 mg/plant and 2.790 mg/plant of tebuconazole commercial formulation Helicur 250 EW. Unlike all the authors listed above, Muñoz-Leoz et al. (2013) did not find a clear trend of changes in dehydrogenase activity under the influence of tebuconazole**.**

Chlorothalonil is highly toxic to dehydrogenase and inhibited the enzyme activity at test concentrations ranging from 0.17 mg/kg to 16.6 mg/kg (Singh et al., 2002; Bending et al., 2007; Baćmaga et al., 2018; Han et al., 2020).

The effect of carbamates macozeb and pirimicarb on dehydrogenase activity is not unambiguous in the studies. Shukla (2000) recorded a slight decrease in enzyme activity at a macozeb concentration of 2.74 mg/kg, while Rasool & Reshi (2010) registered a stimulating effect at many times higher fungicide concentrations (above 800 mg/kg). Probably in the research of Rasool & Reshi (2010) a "stress metabolism" was caused, which is characterized by increased activity of cellular enzymes for generating energy in the affected cells. Alvarez-Martin et al. (2016) observed a slight decrease in dehydrogenase activity at 2.74 mg/kg concentration of pirimicarb.

The benzimidazole fungicide benomyl stimulated dehydrogenase activity both at low (0.51 mg/kg; Shukla, 2000)) and high (51 mg/kg; (Chen et al., 2001)) concentrations. Another benzidimazole, such as carbendazim, had a short-term inhibitory effect on dehydrogenase, followed by enzyme recovery at all tested concentrations (0.76 mg/kg – 100 mg/kg) on 56-th (Burrows & Edwards, 2004) or 90-th (Wang et al., 2016) day after treatment.

Effect of fungicides on soil cellulase

Cellulose is the most common structural polysaccharide in soil and accounts for almost 50% of plant biomass (Eriks-

son et al., 1990). Cellulase is an enzyme that is produced mainly by microorganisms, but is also found in plants and some invertebrates (Sadhu & Maiti, 2013). Cellulase catalyzes the hydrolysis of cellulose to D-glucose (Hussain et al., 2009) and is a complex consisting of at least three enzymes (Joachim & Patrick, 2008): endo-1,4-β-glucanase, which randomly attacks cellulose chains; exo-1,4-β-glucanase, which removes glucose or cellobiose from the non-reducing end of cellulose chains, and β-D-glucosidase, which hydrolyzes cellobiose and other water soluble cellodextrins to glucose (Makoi & Ndakidemi, 2008).

The effects of fungicides on soil cellulase have been studied by a number of authors (Srinivasulu & Rangaswamy, 2006; Floch et al., 2011; Ramudu et al., 2011; Deborah et al., 2013), as they all found similar pattern of changes in enzyme activity under fungicide stress. Low doses (0.2 – 1.0 kg/ha) of propiconazole, tridemorph and captan (Srinivasulu & Rangaswamy, 2006; Ramudu et al., 2011; Deborah et al., 2013) stimulated cellulase immediately after fungicide application, followed by a weak inhibition and enzyme recovery. This trend of changes in cellulase activity was also observed by Floch et al. (2011), using mancozeb at a concentration of 100 mg/kg. Higher concentrations (7.5 kg/ha and 10.0 kg/ha) of propiconazole, tridemorph and captan either did not cause a significant effect (Srinivasulu & Rangaswamy, 2006) or were toxic (Ramudu et al., 2011) to soil cellulase.

Effect of fungicides on soil β*-glucosidase*

β-glucosidase belongs to the group of cellulolytic enzymes that catalyze the hydrolysis of glycosidic bonds of cellulose residues in soil (de Vries & Visser, 2001). The end product of the enzyme's activity is glucose, an important energy source for soil microbial communities. It is known that β-glucosidases are synthesized in plants, animals and microorganisms (Veena et al., 2011). According to some authors, soil β-glucosidase activity is represented mainly by enzymes of microbial origin that are excreted in soil solution or are immobilized on clay or humus colloidal particles (Busto & Perez-Mateos, 2000). It has been identified that β-glucosidase is an enzyme which is sensitive to changes in soil and can be used as an early indicator of changes in the soil organic complex as a result of different agricultural practice (Turner et al., 2002; de la Horra et al., 2003).

There is a relatively small interest in the action of fungicides on soil β-glucosidase. The toxic effects of the triazoles tebuconazole and difenoconazole (Muñoz-Leoz et al., 2011, 2013; Baćmaga et al., 2020), carboxyamide boscalid (Xiong et al., 2014), the imidazole prochloraz (Tejada et al., 2011), and the phenylamide metalaxyl (Sukul, 2006) were studied. According to Muñoz-Leoz et al. (2011, 2013) a concentration of 5 mg/kg difenoconazole had no significant effect on enzyme activity, while higher concentrations of difenoconazole (50 mg/kg and 500 mg/kg), as well as all tested concentrations of tebuconazole (5.0 mg/kg – 500 mg/kg) had a negative effect on β-glucosidase. Sukul (2006), Tejada et al. (2011) and Xiong et al. (2014) found that low tested fungicide concentrations (100 mg/ha metalaxyl, 1 l/ha prochloraz and 10 mg/kg boscalid) had a weak stimulating effect on soil β-glucosidase, and high concentrations (400 mg/ha metalaxyl, 4 l/ha prochloraz and both 100 mg/kg and 200 mg/kg boscalid) inhibited it. Baćmaga et al. (2020) reported insignificant effects of tebuconazole commercial product Helicur 250 EW on β-glucosidase, except at the highest used concentrations of 1.395 mg/plant and 2.790 mg/plant, where the enzyme activity decreased by 5.6% and 7.0%, respectively.

A comparative analysis of the effects of the fungicides listed above showed that the imidazole prochloraz and the triazole tebuconazole had relatively highly toxicity to soil β-glucosidase, which showed an inhibitory effect even at concentrations of 0.5 mg/kg and 0.26 ml/kg (equal to 4 l/ha), respectively.

Effect of fungicides on soil invertase

Invertase (β-fructofuranosidase, E.C.: 3.2.1.5) catalyzes the hydrolysis of sucrose to glucose and fructose – low molecular weight sugars, which are an essential source of energy for microorganisms (Jin et al., 2009). The enzyme is synthesized and released into the soil by microorganisms, plants and some animals (Belcarz et al., 2002), and catalyzes the hydrolysis of sucrose under both acidic and alkaline environmental conditions. Invertase is used as an indicator to assess the efficiency of food and energy metabolism, as well as the efficiency of pollutants' decomposition in soils (Nannipieri et al., 1990).

The triazoles tebuconazole, triadimefon and propiconazole (Ramudu et al., 2011; Deborah et al., 2013; Wang et al., 2016; Sun et al., 2020), and benzimidazole fungicide carbendazim (Yan et al., 2011; Wang et al., 2016; Zhao et al., 2016) are the most commonly tested for effects on invertase. Low concentrations of triazole fungicides stimulated invertase activity, while high (10 mg/kg) and very high (above 100 mg/kg) concentrations inhibited it. Unlike other authors, Deborah et al. (2013) found that triadimefon inhibited invertase activity even at a concentration of 0.2 kg/ha (equal to 2.7 mg/kg).

Like triazoles, carbendazim had a weak stimulating effect on soil invertase at lower concentrations (1.0 mg/kg – 10.0 mg/kg) (Yan et al., 2011; Wang et al., 2016; Zhao et al., 2016), and an inhibitory effect at a concentration of 100 mg/kg (Wang et al., 2016).

The pattern of enzyme stimulation by low and inhibition by high doses was confirmed also for the fungicides mancozeb (Walia et al., 2014), chlorothalonil (Ramudu et al., 2011), tridemorph (Srinivasulu & Rangaswamy, 2006), and captan (Srinivasulu & Rangaswamy, 2006). The stimulating effect was usually weak and was followed by enzyme stabilization within a few months.

Wang et al. (2020) found that azoxystrobin did not influence on soil invertase activity at concentration of 2 mg/kg, and significantly decreased it on day 35 at fungicide concentrations of 25 mg/kg and 50 mg/kg.

Effect of fungicides on soil amylase

Starch is one of the main polysaccharides synthesized by plants and is considered the third source of plant biomass on the planet after lignocelluloses. Amylases are extracellular enzymes that catalyze the hydrolysis of internal α-1,4-glycosidic bonds in starch molecule to dextrin and other small glucose units (Ryan et al., 2006). Based on the site of action, amylase enzymes are classified as: α-amylase, β-amylase and γ-amylase. α-and β-amylases are widespread in the soil as α-amylase is synthesized by plants, animals and microorganisms, while β-amylase is synthesized mainly by plants (Thoma et al., 1971).

It was found that mancozeb (Walia et al., 2014) and triadimefon (Deborah et al., 2013) simulated soil amylase at low doses (mancozeb: 10 ppm and triadimefon: 0.2 kg/ha), while higher doses (mancozeb: 200 – 2000 ppm and triadimefon: 0.5 – 0.7 kg/ha) inhibited the activity of the enzyme. Triadimefon was much more toxic to soil amylase than mancozeb, since even a concentration of 0.5 kg/ha (6.8 mg/kg) inhibited the enzyme. Mancozeb stimulated amylase even at a concentration of 100 ppm, but the effect was temporary and then underwent a process of inhibition.

Effect of fungicides on soil phosphatases

The hydrolysis of organic phosphates in the soil is catalyzed by phosphatases – acidic and alkaline phosphatases, which are activated depending on the environmental pH. Sources of soil acid phosphatase are plant roots (Bull et al., 2002), fungi (Tarafdar & Marschner, 1994) and bacteria (Tarafdar & Claassen, 1988), whereas for alkaline phosphatase – soil microorganisms and invertebrates (Tarafdar & Claassen, 1988). Microbial phosphatases are mainly bound to the cell membrane or are located on the outer surface of the cell wall (Lacaze, 1983).

Among soil phosphatases, the most commonly studied enzymes for soil quality assessment are phosphomonoesterases. Phosphomonoesterases hydrolyze monophosphates with the release of inorganic phosphorus (Reid & Wilson, 1971), but cannot initiate the hydrolysis of phospholipids and nucleic acids without the activity of phosphodiesterases. Initially, the hydrolysis of organic substances is carried out by phosphodiesterase with the release of phosphate monoesters, which are then hydrolyzed by phosphomonoesterase to bioavailable phosphorus.

The fungicide effects were most often evaluated for acid (20 authors tested 20 fungicides) and less for alkaline (14 authors tested 12 fungicides) phosphomonoesterases (Rahmansyah et al., 2009; Cycoń et al., 2010; Rasool & Reshi, 2010; Floch et al., 2011; Madakka et al., 2011; Muñoz-Leoz et al., 2011, 2013; Walia et al., 2014; Saha et al., 2016; Wang et al., 2016; Satapute et al., 2019; Baćmaga et al., 2020; Wang et al., 2020). Only Floch et al. (2011) in their study performed a complete analysis of the effects of mancozeb on phosphatases, following the effects of the fungicide on both mono- (acidic and alkaline), and di- and tri- (involved in the degradation of a number of pesticides) phosphoesterases.

In general, the effects of carbamates (mancozeb, thriam, probineb) on acid (Rahmansyah et al., 2009; Floch et al., 2011; Madakka et al., 2011; Walia et al., 2014) and alkaline (Rasool & Reshi, 2010; Floch et al., 2011) phosphomonoesterases can be reported as stimulating. Only at concentrations above 100 mg/kg, a temporary weak inhibitory effect occured, followed by a rapid recovery of the enzyme activity (Cycoń et al., 2010; Floch et al., 2011; Walia et al., 2014). Floch et al. (2011) also found that mancozeb stimulated di- and tri- phosphosterases.

Triazoles difenococnazole and propiconazole had a stimulating effect on acid phosphatase as during prolonged exposure this effect decreased and even inhibition was registered (Madakka et al., 2011; Satapute et al., 2019). Saha et al. (2016) recorded an exactly reverse trend of the effect of tebuconazole on acid phosphomonoesterase, although they used the same range of concentrations as Madakka et al. (2011) and Satapute et al. (2019). Baćmaga et al. (2020) reported that only the highest used concentration (2.790 mg/plant) of tebuconazole (Helicur 250 EW) reduced significantly the activity of acid phosphomonoesterase, and the reduction rate was 37.5% of the control. In general, higher doses (over 13 mg/kg) of fungicides difenococnazole and propiconazole inhibited acid phosphomonoesterase (Madakka et al., 2011; Satapute et al., 2019). Tebuconazole inhibited alkaline phosphomonoesterase at prolonged exposure (Muñoz-Leoz et al., 2011; Saha et al., 2016; Wang et al., 2016; Baćmaga et al., 2020), although it was reported either no effect (Saha et al., 2016; Wang et al., 2016) or enzyme stimulation (Muñoz-Leoz et al., 2011) immediately after fungicide application. Difenococnazole had no effect on alkaline phosphomonoesterase at a concentration of 5 mg/kg and manifested dose-de-

pendent inhibition at 10- and 100- fold higher concentrations (Muñoz-Leoz et al., 2013).

Phenylamides mefenoxam and metalaxyl did not show a clear effect (metalaxyl**)** or stimulated (mefenoxam) acid phosphomonoesterase activity in a wide range of concentrations (1 mg/kg – 1000 mg/kg) (Monkiedje et al., 2002). Mefenoxam and metalaxyl had no significant effect on alkaline phosphomonoesterase in concentrations up to 200 mg/kg, but the highest tested concentration of 1000 mg/kg resulted in weak (metalaxyl) or much stronger (mefenoxam) inhibition of the enzyme activity.

The strobilurin azoxystrobin significantly decreased the activity of acid phosphomonoesterase by concentrations of 25 mg/kg and 50 mg/kg, and the effect was clearly manifested on day 14 after soil fungicide amendment (Wang et al., 2020). Wang et al. (2020) found that enzyme inhibition occurred even at fungicide concentration of 2 mg/kg, but the effect was delayed (on 35 day after soil amendment) compared to that under the higher used fungicide.

The chloronitrile chlorothalonil was toxic to acid phosphomonoesterase (Singh et al., 2002; Baćmaga et al., 2018), but had no clear defined effect on alkaline phosphomonoesterase (Baćmaga et al., 2018).

Effect of fungicides on soil urease

Urease is an enzyme that catalyzes the hydrolysis of urea to CO_2 and NH_3 (Tabatabai, 1982). It is widespread in nature, synthesized mainly by plants (Polacco, 1977) and microorganisms, and has been found to exist as an intracellular and extracellular form (Mobley & Hausinger, 1989). On the other hand, urease secreted by cells is rapidly deactivated, suggesting that soil urease activity is carried out predominantly by the extracellular form of the enzyme, which is stabilized by immobilization on organic and mineral soil colloids. Urease is a widely used indicator for assessing the impact of agro-ameliorative activities (Saviozzi et al., 2001) or pollutants (Yang et al., 2006) on soil health.

Thiazoles tebuconazole, difenococnazole and propiconazole are the group of fungicides that were the most commonly tested for effects on soil urease (Muñoz-Leoz et al., 2011, 2013; Saha et al., 2016; Wang et al., 2016; Satapute et al., 2019; Baćmaga et al., 2020; Sun et al., 2020) and in a wide range of applied concentrations – from 2.26 mg/kg (Saha et al., 2016) to 500 mg/kg (Muñoz-Leoz et al., 2011, 2013). Saha et al. (2016) found that the recommended field dose (187.5 g/ha) and two-fold higher dose had a stimulating effect on soil urease, while a dose of 1875 g/ha significantly inhibited the enzyme. Sun et al. (2020) confirmed the negative effect of high (10 mg/kg) tebuconazole dose on urease activity. Baćmaga et al. (2020) reported inhibitory effects of Helicur 250 EW on urease activity at concentrations of 1.395 mg/plant and 2.790 mg/plant, and the inhibition rates were 15.6% and 59.9%, respectively.

The above mentioned authors, except Muñoz-Leoz et al. (2013), reported that thiazoles in concentrations above 1 mg/kg had a negative effect on soil urease. Dose-dependent (Wang et al., 2016) or independent (Muñoz-Leoz et al., 2011) effects of tebuconazole on urease at concentrations from 1.0 mg/kg to 500 mg/kg were also found. These differences in the enzyme responses may be explained by soil properties or other environmental characteristics.

The effects of benzimidazoles benomyl and carbendazim on soil urease were studied by Yan et al. (2011), Wang et al. (2016), Zhao et al. (2016) and Shukla (2020). At low concentrations (0.51 mg/kg), benomyl did not cause significant effects on soil urease. According to Yan et al. (2011) and Wang et al. (2016), carbendazim concentrations in the range of 1.0 mg/kg – 100 mg/kg stimulated urease activity with a dose-dependent effect (Wang et al., 2016), which occured at different exposure times. Higher concentrations of carbendazim (340 mg/kg) adversely affected soil urease activity (Zhao et al., 2016).

Strobilurins azoxystrobin and trifloxystrobin were studied in concentrations of 0.075 mg/kg – 50.0 mg/kg (azoxystrobin) and 0.1 – 144 mg/kg (trifloxystrobin). Low concentrations of fungicides did not cause significant effects on soil urease, while concentrations above 10.0 mg/kg had a negative effect on the enzyme (Wightwick et al., 2013; Baćmaga et al., 2015; Guo et al., 2015; Wang et al., 2018; 2020). Usually, enzyme inhibition was recorded later – on the 7-th (Wightwick et al., 2013), 14-th (Guo et al., 2015; Wang et al., 2020) or 30-th (Baćmaga et al., 2015) day after soil treatment with the respective fungicide. Among the above mentioned authors, only Wang et al. (2018) registered urease inhibition immediately after soil amendment with azoxystrobin (0.1 mg/kg – 10.0 mg/kg), followed by an enzyme recovery on day 14. A reversible stimulating effect of 2 mg/kg fungicide concentration on enzyme activity was reported by Wang et al. (2020). Carbamates mancozeb and probineb had a completely inhibitory effect on soil urease, and at the lowest used mancozeb concentration (2.7 mg/kg) the effect was reversible (Shukla, 2000). The enzyme inhibition caused by probineb at concentrations higher than 11.3 mg/kg (Rahmansyah et al., 2009) or by mancozeb in the range of 82.2 mg/kg – 8220 mg/kg (Rasool & Reshi, 2010) was long-term, lasting for probineb more than 12 weeks.

Effect of fungicides on soil arylsulfatase

Soil arylsulfatase is essential for the transformation of sulfur-containing compounds and mineral nutrition of plants.

However, the enzyme has been relatively little studied compared to these involved in carbon, nitrogen and phosphorus cycle. Arylsulfatases are widespread in soils (Gupta et al., 1993) and perform the hydrolysis of soil sulfate esters to phenol and sulfate sulfur (Kertesz & Mirleau, 2004). Arylsulfatases are mainly secreted by bacteria in response to soil sulfur deficiency (Kertesz & Mirleau, 2004), but to a lesser extent they can also be synthesized by plants and animals (Nicholls & Roy, 1971). Extracellular arylsulfatase activity correlates with the amount of soil organic matter and humic acids (Speir & Ross, 1978).

Tebuconazole and difenococnazole have been widely studied in evaluation of arylsulfatase responses to triazole fungicides (Muñoz-Leoz et al., 2011; 2013; Saha et al., 2016; Baćmaga et al., 2020). Saha et al. (2016) found that tebuconazole and difenococnazole applied at concentrations close to the recommended field dose did not cause a significant effect on soil arylsulfatase, but like the other authors, Saha et al. (2016) demonstrated the toxic effect of fungicides when applied at higher concentrations. Baćmaga et al. (2020) did not found any significant effect of Helicur 250 EW (tebuconazole commercial formulation) on arylsulfatase under a wide range of tested fungicide concentrations (0.046 mg/plant – 2.790 mg/plant).

Floch et al. (2011) also reported a negative effect of mancozeb (100 mg/kg) on soil arylsulfatase, which was in line with the above mentioned statements on the negative effects of high fungicides' concentrations on soil enzyme. Sukul (2006) reported a delayed effect of metalaxyl, as low doses (100 g/ha and 200 g/ha) stimulated and high dose (400 g/ha) inhibited arylsulfatase on the 30-th and 60-th day after soil amendment.

Conclusions

Fungicides are extensively used in agriculture as a part of disease control strategies. Fungicides kill or inhibit phytopathogenic fungi and/or their spores, but they may adversely affect soil functioning including nutrient biotransformation in soils. Therefore, the fungicide impact must be well defined in order to use the best agrochemicals for better effectiveness and less side effects. Soil enzymes are often the primary sites of attack by fungicides. Fungicides attack directly by reacting with the enzymes or inhibit the proliferation of soil microorganisms, which are the main producers of soil enzymes.

Based on literature review, we can conclude that: (1) Fungicides Showed clear effects on almost all analyzed soil enzymes; (2) Either positive or negative, fungicide effects on soil enzymes were dependent – dose and/or exposure time, and/or soil properties; (3) Soil dehydrogenase was the most sensitive enzyme to fungicides, and alkaline phosphatase and catalase were the most resistant; (4) Chlorothalonil was the most toxic to soil enzymes, and mancozeb was the least toxic, followed by carbendazim; (5) The manifestation of fungicide toxicity to soil enzymes was in the following order: mancozeb < carbendazim < azoxystrobin ≈ tebuconazole << chlorothalonil.

This review gives new insights on the most appropriate enzymes for bioindication of fungicide impact on soil quality. If fungicides that are not fully analyzed are tested in detail this will enrich the data, and it could be in favor of decision making and accurate fungicide application.

Disclosure of interest

The authors report no conflict of interest.

References

Álvarez-Martín, A., Hilton, S. L., Bending, G. D., Rodríguez-Cruz, M. S. & Sánchez-Martín, M. J. (2016). Changes in activity and structure of flogupthe soil microbial community after application of azoxystrobin or pirimicarb and an organic amendment to an agricultural soil. *Applied Soil Ecology*, *106*, 47–57.

Baćmaga, M., Kucharski, J. & Wyszkowska, J. (2015). Microbial and enzymatic activity of soil contaminated with azoxystrobin. *Environmental Monitoring and Assessment*, *187*, 615.

Baćmaga, M., Wyszkowska, J. & Kucharski, J. (2018). The influence of chlorothalonil on the activity of soil microorganisms and enzymes. *Ecotoxicology*, *27*, 1188–1202.

Baćmaga, M., Wyszkowska, J. & Kucharski, J. (2020). Response of soil microorganisms and enzymes to the foliar application of Helicur 250 EW fungicide on *Horderum vulgare* L. *Chemosphere*, *242*, 125163.

Belcarz, A., Ginalska, G., Lobarzewski, J. & Penel, C. (2002). The novel non-glycosylated invertase from *Candida utilis* (the properties and the conditions of production and purification). *Biochimica et Biophysica Acta*, *1594(1),* 40–53.

Bello, D., Gil-Sotres, F., Leirós, M. C. & Trasar-Cepeda, C. (2012). Laboratory Contamination with 2,4,5-trichlorophenol: Effects on some enzymatic activities in two forest and two agricultural soils of contrasting pH. In: Trasar-Cepeda, C., Hernández, T., García, C., Rad, C. & González-Carcedo, S. (eds.), *Soil enzymology in the recycling of organic wastes and environmental restoration*, Environmental Science and Engineering. *Springer-Verlag,* Berlin Heidelberg, 219-229.

Bending, G. D., Rodríguez-Cruz, M. S. & Lincoln, S. D. (2007). Fungicide impacts on microbial communities in soils with contrasting management histories. *Chemosphere*, *69(1),* 82-88.

Bull, H., Murray, P. G., Thomas, D., Thomas, D., Fraser, A. M. & Nelson, P. N. (2002). Acid phosphatases. *Molecular Pathology*, *55(2),* 65–72.

Burrows, L. A. & Edwards, C. A. (2004). The use of integrated

soil microcosms to assess the impact of carbendazim on soil ecosystems. *Ecotoxicology*, *13*, 143–161.

Busto, M. D. & Perez-Mateos, M. (2000). Characterization of β-d-glucosidase extracted from soil fractions. *European Journal of Soil Science*, *51(2),* 193-200.

Chen, S. K., Edwards, C. A. & Subler, S. (2001). Effects of the fungicides benomyl, captan and chlorothalonil on soil microbial activity and nitrogen dynamics in laboratory incubations. *Soil Biology and Biochemistry*, *33(14),* 1971-1980.

Cycoń, M., Piotrowska-Seget, Z. & Kozdrój, J. (2010). Responses of indigenous microorganisms to a fungicidal mixture of mancozeb and dimethomorph added to sandy soils. *International Biodeterioration & Biodegradation*, *64(4),* 316-323.

Dardis, J. & Walsh, E. J. (2000). Studies on the effectiveness of metconazole in controlling Fusarium head blight caused by *Fusarium culmorum* in spring wheat (*Triticum aestivum* L). *Cereal Research Communications*, *28*, 443–448.

de la Horra, A. M., Conti, M. E. & Palma, R. M. (2003). β-Glucosidase and proteases activities as affected by long-term management practices in a typic argiudoll soil. *Communications in Soil Science and Plant Analysis*, *34*, 2395–2404.

de Vries, R. P. & Visser, J. (2001). Aspergillus enzymes involved in degradation of plant cell wall polysaccharides. *Microbiology and Molecular Biology Reviews*, *65(4),* 497–522.

Deborah, B. V., Mohiddin, M. J. & Madhuri, R. J. (2013). Interaction effects of selected pesticides on soil enzymes. *Toxicology International*, *20(3),* 195-200.

Dow AgroSciences (2008). Internal Market Research Based On Panel Data Supplied by Agrobase-Logigram. Archamps, France.

Eriksson, K. E. L., Blancbette, R. A. & Ander, P. (1990). Biodegration of Cellulose. In: Eriksson, K. E. L., Blanchette, R.A. & Ander, P. (eds.), *Microbial and Enzymatic Degradation of Wood and Wood Components*. Springer, New York, 89-180.

Floch, C., Chevremont, A., Joanico, K., Capowiez, Y. & Criquet, S. (2011). Indicators of pesticide contamination: Soil enzyme compared to functional diversity of bacterial communities via Biolog® Ecoplates. *European Journal of Soil Biology*, *47(4),* 256–263.

Garthwaite, D. G., Thomas, M. R., Dawson, A. & Stoddart, H. (2002). Arable crops in Great Britain. *Pesticide Usage Survey Report*, *187*, 1–108.

Gooding, M. J. & Davies, P. W. (1997). Wheat Production and Utilization: Systems, Quality and the Environment. New York, USA: *CAB Internationals.*

Grand View Research (2020). Fungicides market size, share & trends analysis report by product (inorganic, biofungicides), by application (cereals & grains, fruits & vegetables), by region, and segment forecasts, 2020 – 2027. https://www.grandviewresearch.com/industry-analysis/fungicides-market

Gu, Y., Wag, P. & Kong, C. (2009). Urease, invertase, dehydrogenase and polyphenoloxidase activities in paddy soils influenced by allelophatic rice variety. *European Journal of Soil Biology*, *45*, 436-441.

Gullino, M. L., Tinivella, F., Garibaldi, A., Kemmitt, G. M., Bacci, L. & Sheppard, B. (2010). Mancozeb: Past, present and future. *Plant Disease, 94(9),* 1076-1087.

Guo, P., Zhu, L., Wang, J., Wang, J., Xie, H. & Lv, D. (2015). Enzymatic activities and microbial biomass in black soil as affected by azoxystrobin. *Environmental Earth Sciences*, *72(2),* 1353–1361.

Gupta, V. V. S. R., Farrell, R. E. & Germida, J. J. (1993). Activity of arylsuphatases in Saskatchewan soils. *Canadian Journal of Soil Science*, *73(3),* 341-347.

Han, L., Liu, Y., Fang, K., Zhang, X., Liu, T., Wang, F. & Wang, X. (2020). Dissipation of chlorothalonil in the presence of chlortetracycline and ciprofloxacin and their combined effects on soil enzyme activity. *Environmental Science and Pollution Research*, *27*, 13662–13669.

Hussain, S., Siddique, T., Saleem, M., Arshad, M. G. & Khalid, A. (2009). Impact of pesticides on soil microbial diversity, enzymes, and biochemical reactions. *Advances in Agronomy*, *102*, 160-190.

Jin, K., Sleutel, S., Buchan, D., De Neve, S., Cai, D. X., Gabriels, D. & Jin, J. Y. (2009). Changes of soil enzyme activities under different tillage practices in the Chinese Loess Plateau. *Soil and Tillage Research*, *104(1),* 115-120.

Joachim, H. J. & Patrick, A. N. (2008). Selected soil enzymes: Examples of their potential roles in the ecosystem. *African Journal of Biotechnology*, *7(3),* 181-191.

Kertesz, M. A. & Mirleau, P. (2004). The role of soil microbes in plant sulphur nutrition. *Journal of Experimental Botany*, *55(404),* 1939-1945.

Köhler, H. R. & Triebskorn, R. (2013). Wildlife ecotoxicology of pesticides: Can we track effects to the population level and beyond? *Science*, *341(6147),* 759-765.

Lacaze, B. (1983). Cytochemical distribution of acid phosphatase activities of mycorrhizal fungi grown on media with or without phosphate deficiency. *Canadian Journal of Botany*, *61(5),* 1411-1414, (FR).

Madakka, M., Mohiddin, G. J., Srinivasulu, M. & Rangaswamy, V. (2011). Influence of pesticides, alone and in combination, on phosphatase activity in soils of groundnut (*Arachis hypogaea* L.) fields. *Dynamic Soil, Dynamic Plant 5*, (Special Issue 1), 70-74.

Makoi, J. H. J. R. & Ndakidemi, P. A. (2008). Selected soil enzymes: examples of their potential roles in the ecosystem. *African Journal of Biotechnology*, *7(3),* 181-191.

Mobley, H. L. T. & Hausinger, R. P. (1989). Microbial urease: Significance, regulation and molecular characterization. *Microbiological Reviews*, *53(1),* 85-108.

Monkiedje, A., Ilori, M. O. & Spiteller, M. (2002). Soil quality changes resulting from the application of the fungicides mefenoxam and metalaxyl to a sandy loam soil. *Soil Biology and Biochemistry*, *34*, 1939-1948.

Muñoz-Leoz, B., Garbisu, C., Charcosse, J. Y., Sánchez-Pérez, J. M., Antigüedad, I. & Ruiz-Romera E. (2013). Non-target effects of three formulated pesticides on microbially-mediated processes in a clay-loam soil. *Science of the Total Environment*, *449*, 345–354.

Muñoz-Leoz, B., Ruiz-Romera, E., Antigüedad, I. & Garbisu, C. (2011). Tebuconazole application decreases soil microbial biomass and activity. *Soil Biology and Biochemistry*, *43(10),* 2176–2183.

Nannipieri, P., Grego, S. & Ceccanti, B. (1990). Ecological significance of the biological activity in soil. In: Bollag, J. M. & Stotzky, G. (eds.), *Soil Biochemistry*, *6*, *Marcel Dekker,* New York, 293–355.

Nicholls, R. G. & Roy, A. R. (1971). Arylsulfatase. In: Boyer, P. D. (ed.), *The Enzymes*. vol. 5, 3rd edn., *Academic Press,* New York.

Polacco, J. C. (1977). Is nickel a universal component of plant ureases? *Plant Science Letters*, *10*, 249–255.

QYResearch (2018). Global azoxystrobin market 2018 – industry production, trends, sales, supply, demand, analysis & forecast to 2025. Industry Research Biz.

Rahmansyah, M., Antonius, S. & Sulistinah, N. (2009). Phosphatase and urease instability caused by pesticides in soil improved by grounded rice straw. *ARPN Journal of Agricultural and Biological Science*, *4(2),* 56–62.

Ramudu, A. C., Mohiddin, G. J., Srinivasulu, M., Madakka, M. & Rangaswamy, V. (2011). Impact of fungicides chlorothalonil and propiconazole on microbial activities in groundnut (*Arachis hypogaea* L.) soils. *ISRN Microbiology*, *2011*, 1–7.

Rasool, N. & Reshi, Z. A. (2010). Effect of the fungicide Mancozeb at different application rates on enzyme activities in a silt loam soil of the Kashmir Himalaya, India. *Tropical Ecology*, *51*, 199-205.

Regulation 2019/677 (2019). Commission implementing Regulation (EU) 2019/677, concerning the non-renewal of the approval of the active substance chlorothalonil, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market, and amending Commission Implementing Regulation (EU) No 540/2011. Official Journal of the European Union. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32019R0677&from=EN

Reid, T. W. & Wilson, I. B. (1971). *E. coli* alkaline phosphatase. In: Boyer, P. D. (ed.), *The Enzymes*, Vol. 4, New York, USA7 *Academic Press.*

Report Hive Research (2018). Chlorothalonil market report/ Insights, trends, outlook, and opportunity analysis and forecasts. Slideshare. https://www.slideshare.net/Mike01Ross/chlorothalonil-market-report-analysis-type-demand-and-growth-opportunities-by-report-research.

Ryan, S. M., Fitzgerald, G. F. & van Sinderen, D. (2006). Screening for and identification of starch-, amylopectin-, and pullulan-degrading activities in bifidobacterial strains. *Applied and Environmental Microbiology*, *72(8),* 5289-5296.

Sadhu, S. & Maiti, T. K. (2013). Cellulase production by bacteria: A Review. *British Microbiology Research Journal*, *3(3),* 235-258.

Saha, A., Pipariya, A. & Bhaduri, D. (2016). Enzymatic activities and microbial biomass in peanut field soil as affected by the foliar application of tebuconazole. *Environmental Earth Sciences*, *75*, 558.

Satapute, P., Kamble, M. V., Adhikari, S. S. & Jogaiah, S. (2019). Influence of triazole pesticides on tillage soil microbial populations and metabolic changes. *Science of the Total Environment*, *651*, 2334-2344.

Saviozzi, A., Levi-Minzi, R., Cardelli, R. & Riffaldi, R. (2001). A comparison of soil quality in adjacent cultivated, forest and native grassland soils. *Plant and Soil*, *233*, 251-259.

Shukla, A. K. (2000). Empact of fungicides on soil microbial population and enzyme activity. *Acta Botanica Indica*, *28*, 85-87.

Singh, B., Walker, A. & Wright, D. J. (2002). Degradation of chlorpyrifos, fenamiphos, and chlorothalonil alone and in combination and their effects on soil microbial activity. *Environmental Toxicology and Chemistry*, *21(12),* 2600-2605.

Sopeña, F. & Bending, G. D. (2013). Impacts of biochar on bioavailability of the fungicide azoxystrobin: a comparison of the effect on biodegradation rate and toxicity to the fungal community. *Chemosphere*, *91(11),* 1525-1533.

Speir, T. W. & Ross, D. J. (1978). Soil phosphatase and sulphatase. In: Burns, R. G. (ed.), *Soil Enzymes*. Academic, London, UK.

Srinivasulu, M. & Rangaswamy, V. (2006). Activities of invertase and cellulase as influenced by the application of tridemorph and captan to groundnut (*Arachis hypogaea*) soil. *African Journal of Biotechnology*, *2(5),* 175–180.

Subhani, A., Changyong, H., Zhengmiao, Y., Min, L. & El-ghamry, A. M. (2001). Impact of soil environment and agronomic practices on microbial/ dehydrogenase enzyme activity in soil. A review. *Pakistan Journal of Biological Sciences*, *4*, 333-338.

Sukul, P. (2006). Enzymatic activities and microbial biomass in soil as influenced by metalaxyl residues. *Soil Biology and Biochemistry*, *38*, 320–326.

Sun, T., Miao, J., Saleem, M., Zhang, H., Yang, Y. & Zhang, Q. (2020). Bacterial compatability and immobilization with biochar improved tebuconazole degradation, soil microbiome composition and functioning. *Journal of Hazardous Materials*, *398*, 122941.

Tabatabai, M. A. (1982). Soil Enzyme. In: Page, A. L., Miller, E. M. & Keeney, D. R. (eds.), *Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties*. American Society of Agronomy, Madison, WI.

Tarafdar, J. C. & Claassen, N. (1988). Organic phosphorus compounds as a phosphorus source for higher plants through the activity of phosphatases produced by plant roots and microorganisms. *Biology and Fertility of Soils*, *5(4),* 308–312.

Tarafdar, J. C. & Marschner, H. (1994). Phosphatase activity in the rhizosphere and hyphosphere of VA mycorrhizal wheat supplied with inorganic and organic phosphorus. *Soil Biology and Biochemistry*, *26(3),* 387–395.

Tejada, M., Gómez, I., García-Martínez, A. M., Osta, P. & Parrado, J. (2011). Effects of prochloraz fungicide on soil enzymatic activities and bacterial communities. *Ecotoxicology and Environmental Safety*, *74*, 1708–1714.

Thoma, J. A., Rao, G. V. K., Brothers, C., Spradlin, J. & Li, L. H. (1971). Subsite mapping of enzymes. Correlation of product patterns with Michaelis parameters and substrate-induced strain. *Journal of Biological Chemistry*, *246(25),* 5621-5635.

Turner, B. L., Hopkins, D. W., Haygarth, P. M. & Ostle, N. J. (2002). β-Glucosidase activity in pasture soils. *Applied Soil Ecology*, *20*, 157–162.

Veena, V., Poornima, P., Parvatham, R. & Kalaiselvi, K. (2011). Isolation and characterization of β -glucosidase producing bac-

teria from different sources. *African Journal of Biotechnology*, *10(66),* 14891-14906.

Walia, A., Mehta, P., Guleria, S., Chauhan, A. & Shirkot, C. K. (2014). Impact of fungicide mancozeb at different application rates on soil microbial populations, soil biological processes and enzyme activities in soil. *The Scientific World Journal*, *2014*, 702909.

Wang, C., Wang, F., Zhang, Q. & Liang, W. (2016). Individual and combined effects of tebuconazole and carbendazim on soil microbial activity. *European Journal of Soil Biology*, *72*, 6–13.

Wang, F., Li, X., Zhu, L., Du, Z., Zhang, C., Wang, J., Wang, J. & Lv, D. (2018). Responses of soil microorganisms and enzymatic activities to azoxystrobin in Cambisol. *Polish Journal of Environmental Studies*, *27*, 2775-2783.

Wang, X., Lu, Z., Miller, H., Liu, J., Hou, Z., Liang, S., Zhao, X., Zhang, H. & Borch, T. (2020). Fungicide azoxystrobin induced changes on the soil microbiome. *Applied Soil Ecology*, *145*, 103343.

Wightwick, A. M., Reichman, S. M., Menzies, N. W. & Allinson, G. (2013). The effects of copper hydroxide, captan and trifloxystrobin fungicides on soil phosphomonoesterase and urease activity. *Water, Air & Soil Pollution*, *224*, 1703.

Wood, A. (2021). Compendium of Pesticide Common Names. Fungicides. Copyright © 1995–2021. http://www.alanwood.net/pesticides/class_fungicides.html.

Xiong, D., Li, Y., Xiong, Y., Li, X., Xiao, Y., Qin, Z. & Xiao, Y. (2014). Influence of boscalid on the activities of soil enzymes and soil respiration. *European Journal of Soil Biology*, *61*, 1–5.

Yan, H., Wang, D., Dong, B., Tang, F., Wang, B., Fang, H. & Yu, Y. (2011). Dissipation of carbendazim and chloramphenicol alone and in combination and their effects on soil fungal:bacterial ratios and soil enzyme activities. *Chemosphere*, *84(5),* 634–641.

Yang, Z., Liu, S., Zheng, D. & Feng, S. (2006). Effects of cadmium, zinc and lead on soil enzyme activities. *Journal of Environmental Sciences*, *18*, 1135–1141.

Zhang, N., He, X., Gao, Y., Li, Y., Wang, H., Ma, D., Zhang, R. & Yang, S. (2010). Pedogenic carbonate and soil dehydrogenase activity in response to soil organic matter in *Artemisia ordosica* community. *Pedosphere*, *20*, 229235.

Zhang, N. (2017). QYR: Global Chlorothalonil Market Overview. http://www.chemicalforums.com/ index.php?topic=92197.0.

Zhang, D., Wu, Y., Zhang, X. & Zhu, Y. (2017). Persistence of myclobutanil and its impact on soil microbial biomass C and dehydrogenase enzyme activity in tea orchard soils. *Eurasian Journal of Soil Science*, *6(2),* 106 – 113.

Zhao, S., Chen, X., Deng, S., Dong, X., Song, A., Yao, J., Fang, W. & Chen, F. (2016). The effects of fungicide, soil fumigant, bio-organic fertilizer and their combined application on chrysanthemum *Fusarium* wilt controlling, soil enzyme activities and microbial properties. *Molecules*, *21(4),* 526.

Zubrod, J. P., Bundschuh, M., Arts, G., Brühl, C. A., Imfeld, G., Knäbel, A., Payraudeau, S., Rasmussen, J. J., Rohr, J., Scharmüller, A., Smalling, K., Stehle, S., Schulz, R. & Schäfer, R. B. (2019). Fungicides: an overlooked pesticide class? *Environmental Science & Technology*, *53*, 3347–3365.

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