CHAPTER

# 10

# Fungicide and pesticide fallout on aquatic fungi

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## 1 Introduction

The supply of food and clothing has always been one of the fundamental human needs, and the purpose of agriculture is to supply the same. Due to the expansion of human societies and their increasing needs, agriculture production should be increased accordingly. The world's population was estimated at 2.6 billion in 1950, 5 billion in 1987, and 7.9 billion in 2021 (Worldometers, 2021). At the same rate, the world's population is predicted to be 9.7 billion by 2050 and 21 billion by 2100 (United Nations, 2019a). Accordingly, to supply enough food for the expected 9.6 billion people, it's essential to increase agricultural production by 70% (United Nations, 2013) and water withdrawals by 15% (World Bank, 2017). As a step in this direction, food production has tripled since 1960 as the world's population doubled (OECD, 2018).

It seems that the agricultural land needs to be developed to produce enough food (Fitton et al., 2019). Though the World Bank (2018) reported that the level of agricultural land in the world in 2016 compared to 1990 has increased by approximately 19.5% (Fig. 10.1). But the expansion of agricultural land has caused limitations such as soil pollution and salinization, water shortage, desertification, deforestation, land degradation, soil erosion, etc. Further, approximately 10–11% of the world's agricultural land is vulnerable to water scarcity, especially in Africa, the Middle East, China, Asia, and Europe Fitton et al. (2019).

In addition to land restriction and water scarcity, agricultural production faces other significant challenges such as plant diseases, weeds, and pests (Swaroopa Rani, Nadendla, Bardhan, Madhuprakash, & Podile, 2020). Plant diseases and pests are major threats to biosafety (Rolfe,

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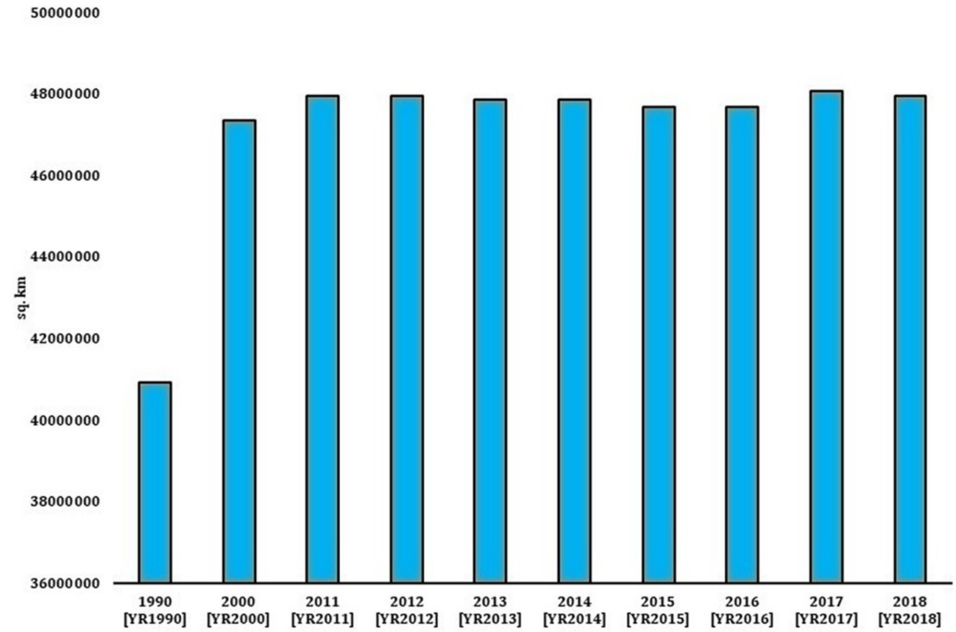


**FIG. 10.1** The extension of agricultural land in the world from 1990 to 2018. *From World Bank. (2018). Agricultural Land (% of Land Area). https://data.worldbank.org/indicator/AG.LND.AGRI.ZS?end=2018&start=1961&view=chart.*

2006), e.g., Savary, Ficke, Aubertot, and Hollier (2012) concluded that the harmful effects of plant diseases on food processing and the safety of many crops worldwide are serious. Therefore, farmers use large amounts of fertilizers and pesticides to increase high-yielding plants (Shefali, Kumar, Sankhla, Kumar, & Sonone, 2021).

The pesticides that generally kill various pests include rodenticides, herbicides, insecticides, nematicides, fungicides, bactericides, etc. (Aktar, Sengupta, & Chowdhury, 2009). Approximately 2 million tons of pesticides are consumed annually worldwide, and by 2020, the world's pesticide use was 3.5 million tonnes (Sharma et al., 2019). The quantities (in tonnes of active ingredients) of fungicides and bactericides used in or sold to the agricultural sector for crops and seeds are shown in Fig. 10.2.

With the advancement of technology and modern insecticides, herbicides, and fungicides, the risk of plant diseases has been reduced (Ahmed & Kumar, 2020). Among all the plant pathogens, fungi are one of the most important harmful agents. Fungi have evolved over a billion years, and due to their high adaptability, they can be found in various habitats (Tleuova et al., 2020). Fungal pathogens cause great damage to fruits during storage and transportation (Zhang, Li, Zhang, Chen, & Tian, 2020). For example, *Botrytis cinerea* causes significant economic damage to grapes, vegetables, and berry crops worldwide (Rosslenbroich & Stuebler, 2000). It is one of the most important agricultural phytopathogens and the cause of gray mold in more than one thousand plant species (Islam & Sherif, 2020). Zhang, Godana, et al. (2020) also stated that gray and blue mold diseases are among the most damaging grape diseases worldwide. The economic losses by *B. cinerea* are estimated at more than ten billion dollars

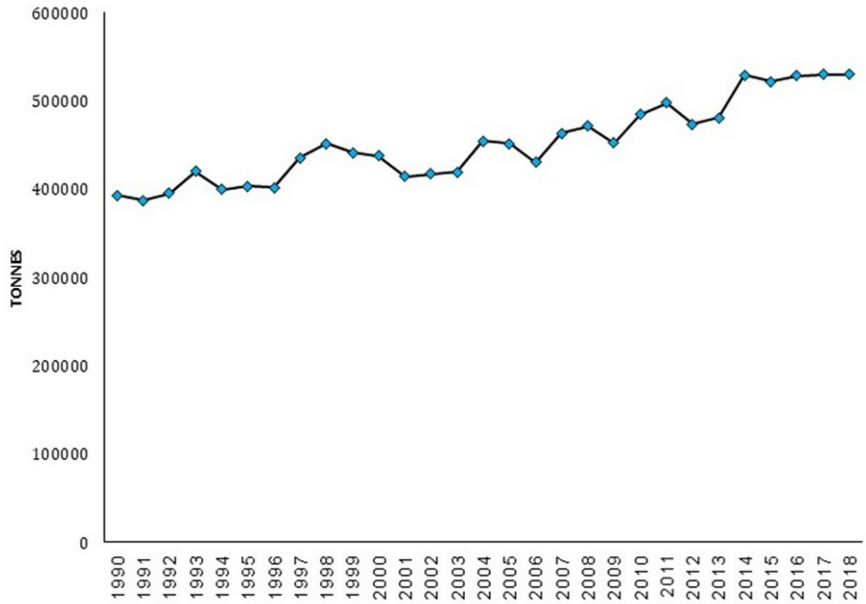


**FIG. 10.2** The world quantities (in tones of active ingredients) of fungicides and bactericides from 1990 to 2018. *From FAO. (2021). http://www.fao.org/faostat/en/#search/fungicides%20and%20bactericides.*

annually (Petrasch, Knapp, van Kan, & Blanco-Ulate, 2019). Anthracnose is also a fungal disease that causes significant damage to crop production (Nasran, Mohd Yusof, Halim, & Abdul Rahman, 2020). Phytopathogenic microorganisms reduce crop yields and their commercial quality (Khakimov, Omonlikov, & Utaganov, 2020). According to Dukare, Singh, Jangra, and Bhushan (2020), the loss of post-harvest crops due to rot caused by pathogenic fungi is a cause of significant economic loss. In addition to crop damage, *Penicillium* spp. is involved in the production of mycotoxins, alkaloids, and allergens, which negatively affect human health. A mycotoxin is a potentially toxic chemical produced by fungi in food and is dangerous to humans (Taylor & Baumert, 2014).

Fungicides are the primary tool for plant disease management, and farmers are heavily dependent on chemical fungicides to prevent crop failure due to fungal diseases (McGrath, 2009; Ons, Bylemans, Thevissen, & Cammue, 2020). The fungicide mode of action is based on stopping the activity of enzymes and inhibiting signal transmission (Yamaguchi & Fujimura, 2005). There are two major classes of fungicides: (i) Bordeaux inorganic mixtures and sulfur and copper compounds; (ii) phthalimides, dithiocarbamates, dinitrophenols, and aromatic hydrocarbons. These agrochemicals are used only for high-yielding plants due to their high financial value (Fernández-Ortuño, Torés, De Vicente, & Pérez-García, 2008). Fungicides are released into the air and destroy fungal pathogens responsible for crop damage (Biswas, Sarkar, & Singh, 2020). Biochemical studies of Botryticides have shown that anilinopyrimidines inhibit methionine biosynthesis by blocking cystathionine beta-lyase (Rosslenbroich & Stuebler, 2000). Dithiocarbamate (DTF) are non-systemic and have been implemented to prevent several fungal diseases in plants since the 1940s (Fanjul-Bolado, Fogel, Limson, Purcarea, & Vasilescu, 2021). The main fungicides, carbendazim, thiabendazole, and triazole, are also widely applied to prevent fungal diseases in vegetables and fruits (Zhao et al., 2020; Senosy, Guo, et al., 2020).

Maneb, mancozeb, metiram, ziram, thiram, Na-dimethyldithiocarbamate, ferbam, and metam sodium are the most commonly used dithiocarbamate pesticides, which inhibit the metal-dependent and sulfhydryl enzyme systems in microorganisms (e.g., fungi and bacteria), plants, insects, and mammals. Dithiocarbamate results in neuropathology, thyroid toxicity, and central nervous system developmental toxicity (US EPA, 2001).

Phthalimides are a class of non-aromatic nitrogen cycle heterocycles that have a wide range of applications. Accordingly, they are used for antifungal and antibacterial activity (Kushwaha & Kaushik, 2016). Phthalimides include captan, captafol, and folpet fungicides, used as surface protectors for crops and are not usually toxic to mammals (Gupta & Aggarwal, 2007).

Currently, six different isomers of dinitrophenols (e.g., 2,3-DNP, 2,4-DNP, 2,5-DNP, 2,6-DNP, 3,4-DNP, and 3,5-DNP) are available and the most important among them is the 2,4-DNP isomer. One of the applications of dinitrophenols is their use in fungicides, herbicides, and insecticides. Interrupting oxidative phosphorylation via separating the electrochemical gradient in the mitochondrial membrane is known as their mechanism of action. Therefore, they can be highly toxic to humans and animals (Janz, 2005). One of the famous types of dinitrophenyl, which has been used since the 1930s, is the dinocap fungicide. It is a complex combination of 2,4-dinitro-6-octylphenyl and 2,6-dinitro-4-octylphenyl. The octyl part is also a mixture of 1-methylheptyl, 1-ethylhexyl, and 1-propylpentyl isomers. Crotonate, Karathane, Sialite, Caprane, Crotothane, Mildane, and Mildex are the commercial names for this fungicide (Hollingworth, 2001). Table 10.1 summarizes studies on the control of plant diseases by chemical fungicides.

Although fungicides have significantly helped increase productivity in agriculture, their overuse has led to substantial environmental concerns (Tleuova et al., 2020). The release of heavy metals from fungicides and other pesticides in the environment has posed long-term risks to human health and ecosystems (El-Nagar, Massoud, & Ismail, 2020). Thus, many studies have been conducted on the growing concerns of pesticide residues in the environment, food, and feed in recent years. Therefore, the consumption of some chemical fungicides has been limited (Mihajlovic et al., 2020). Although modern fungicides are produced safely, there is a significant concern about their side effects on non-target organisms (Yamaguchi & Fujimura, 2005). Strobilurin fungicides (SF) are a new class of chemical fungicides that have been generally used in agricultural fields for decades (Feng, Huang, Zhan, Bhatt, & Chen, 2020). In a study, SF toxicity on aquatic and soil organisms was studied (Zhang, Zhou, et al. (2020)) and was found to be highly toxic to aquatic and terrestrial organisms. Limited studies have been conducted on the effects of chemical fungicides on aquatic and terrestrial ecosystems (da Conceição Marinho, Diogo, Lage, & Antunes, 2020).

## 2 Pesticides in aquatic ecosystems

### 2.1 Fungicides and water resources contamination

The availability of clean and safe drinking water is considered a fundamental human right (United Nations, 2019b). Safe and readily available water is essential for public health, whether used for domestic, food production or recreational purposes (WHO, 2019). Global

**TABLE 10.1** Key fungicides for plant disease control in different regions of the world.

| Plant | Disease | Agent | Fungicides | Region | References |
|---|---|---|---|---|---|
| Cucumber | Downy mildew | *Pseudoperonospora cubensis* | Oxathiapiprolin, Cyazofamid, Propamocarb, Ethaboxam, Fluazinam and a mixture of mancozeb and zoxamid | North California, United States | D'Arcangelo, Adams, Kerns, and Quesada-Ocampo (2021) |
| Mandarin orange | Phytophthora | *Phytophthora palmivora* | Potassium phosphonate, Metalaxyl-M, Mancozeb | North Vietnam | Chi et al. (2020) |
| Rubber tree | Leaf fall | *Neopestalotiopsis cubana* | Prochloraz, Metalaxy, Carbendazim, Hexaconazole, Mancoxeb, Captan, Aironae, Propineb, Copper oxychloride, etc. | South Thailand | Thaochan, Pornsuriya, Chairin, and Sunpapao (2020) |
| Onion | Stemphylium leaf blight | *Stemphylium vesicarium* | Azoxystrobin, Pyrimethanil | Ontario, Canada | Stricker, Tayviah, Gossen, and Mcdonald (2020) |
| Rice | Rice blast and dirty panicle | *Pyricularia oryzae*, *Bipolanis oryzae*, *Curvularia lunata*, *Fusarium incarnatum* | Mancozeb, Fluopyram, Tebuconazole, Carbendazim, Flutriafol, Triazole, Strobilurin, Azoxystrobin, Thiophanate-metyl | Thailand | Kongcharoen, Kaewsalong, and Dethoup (2020) |
| Sunflower | Seed-borne fungi disease | *Alternaria* spp., *Fusarium* spp., *V. dahliae*, *Cladosporium cladopsporiodes* | Carbendazim, Triadimefon, Flusilazole | China | Addrah et al. (2020) |
| Wheat and Barley | Septoria tritici blotch, Tan spot, Stagonospora nodorum blotch | *Zympseptoria tritici*, *Pyrenophora tritici-repentis*, *Parastagonospora nodorum* | A collection of agrochemical fungicides | Nordic-Baltic (Europe) | Jalli et al. (2020) |
| Wheat | Stripe rust | *Puccinia striiformis* | A collection of agrochemical fungicides | – | Carmona, Sautua, Pérez-Hérnandez, and Reis (2020) |
| Poplar tree | Seedling blight | *Sirococcus conigenus* | Fludioxonil, Difenoconazole | Norway | Brodal et al. (2020) |

*Continued*

**TABLE 10.1** Key fungicides for plant disease control in different regions of the world—cont'd

| Plant | Disease | Agent | Fungicides | Region | References |
|---|---|---|---|---|---|
| *Vigna radiate* L. and *Vigna mungo* L. | Charcoal rot | *Macrophomina phaseolina* | Benomyl, Carbendazim, Copper oxychloride, Mancozeb, Propineb | Pakistan | Iqbal and Mukhtar (2020) |
| Sunflower | Alternaria leaf blight | *Alternaria alternata* | Difenonazole, Fluquinconazole, Metalaxyl-M, Fludioxonil, Azoxystrobin, Epoxyconazole | South Africa | Kgatle, Flett, Truter, and Aveling (2020) |
| Cocoa | Black pod | *Phytophthora palmivora, Phytophthora megakarya* | A collection of agrochemical fungicides | Ghana | Oduro, Apenteng, and Nkansah (2020) |
| Onion | Downy mildew | *Peronospora destructor* | Metalaxyl-M, Mancozeb | Southern Brazil | Araújo, Resende, Alves, and Higashikawa (2020) |

water use has grown more than double the population growth rate since the last century (United Nations, 2018). Access to water resources in the world has increasingly limited due to the contamination of freshwater resources by the disposal of untreated wastewater in lakes, rivers, and groundwater resources (UNESCO, 2019).

Water contamination is an essential factor in threatening water security, especially in semi-arid and arid regions. In addition to water resources restriction, industrialization, and anthropogenic activities can have a devastating effect on the water quality on the Earth (Ma et al., 2020). Contamination of freshwater ecosystems by different kinds of pollutants (heavy metals, pharmaceuticals, and pesticides) is a global environmental issue, e.g., several herbicides such as metribuzin, dacthal, terbuthylazine, tebuthiuron, simazine, and fungicides azoxystrobin, thiabendazole, epoxiconazole, propiconazole metalaxyl, carbendazim, and pyrimethanil were reported to be present in the South African freshwater (Barnhoorn & van Dyk, 2020). Similarly, insecticides, herbicides, and fungicides were found with a concentration of more than $1\,\mu g\,L^{-1}$ (exceeds the EU standard for humans) in the Spanish freshwater (Herrero-Hernández, Simón-Egea, Sánchez-Martín, Rodríguez-Cruz, & Andrades, 2020).

The quantity of pesticides in the water depends on drainage, rainfall, microbial activity, soil, and pesticides physicochemical parameters (temperature, treatment surface, solubility, mobility, and half-life) and their application rates (Agrawal, Pandey, & Sharma, 2010; Hossain et al., 2015). Water contaminated with pesticides, especially fungicides, has dangerous consequences for human health (Senosy, Lu, et al., 2020). Fungicides are less studied than insecticides and herbicides (Zubrod et al., 2019). Surface runoff and leaching further transfer the pesticides and their residues to groundwater (Stevenson, Baumann, & Jackman, 1997). There is no doubt that pesticides reduce water quality, especially in drinking water resources (Syafrudin et al., 2021).

Ramírez-Morales et al. (2021) investigated the effects of 42 pesticides, including 2 types of herbicides, 4 types of insecticides, and 2 types of fungicides, on water quality in Costa Rica and observed 9 pesticides in the micro-catchments, with 2 pesticides exceeding the critical ecotoxicological concentration and 4 exceeding the limit value for at least 1 international guideline. Similarly, concentrations of 162 pesticides were found in water resources and drinking water of paddy fields in Japan by Kamata, Matsui, and Asami (2020), indicating that the herbicides and fungicides used in rice cultivation are often detected in drinking water resources. Pesticides leaching to groundwater and runoff to surface water poses a huge threat to water security and risk to the environment and food safety (Bernardes, Pazin, Pereira, & Dorta, 2015; de Souza et al., 2020). The effects of pesticides on water resources are summarized in Table 10.2.

**TABLE 10.2** Several studies conducted on contamination of aquatic ecosystems in different regions with pesticides.

| The active substance of pesticide | Region | Effect | Reference |
|---|---|---|---|
| DDT, DDE, atrazine, Beta-cyfluthrin | Three bayous in Mississippi, United States | Residues of pesticides in sediments and amphibians body (*Hyalella azteca*) | Lizotte, Steinriede, and Locke (2021) |
| (Atrazine, deethyl-atrazine, simazine); fungicides: (carbendazim, tebuconazole, epoxiconazole); insecticides: (imidacloprid) | An agricultural watershed, southern Brazil | The presence of five herbicides in maize and forage | de Castro Lima et al. (2020) |
| (Imidacloprid, thiacloprid, chlorpyrifos, acetamiprid); herbicide: (terbuthylazine) | Three watersheds, South Africa | The presence of pesticides in the water flows from the farm to the watersheds | Curchod et al. (2020) |
| Atrazine, acetochlor | Drinking water resources in Dalian, China | The presence of heavy metals (Hg and Cr) caused by pesticides in water samples and soil sediments | Dong, Zhang, and Quan (2020) |
| Chlorpyrifos, diazinon, auinalphos | Linggi River, Malaysia | High risk of pesticides in aquatic ecosystems | Zainuddin, Wee, and Aris (2020) |
| (Tebuconazole, carbendazim, azoxystrobin); herbicides: (atrazine, dicamba, 2,4-D) | Two watersheds in the Cordoba province and a watershed in the Buenos Aires province, Argentina | The presence of pesticides in agricultural runoff and outflows from urban areas, reduction of water quality | Corcoran, Metcalfe, Sultana, Amé, and Menone (2020) |
| Chlorpyrifos | Iberian, Peninsula, Spain and Portugal | High eco-toxicological risk of pesticides in surface water ecosystems | Rico, Dafouz, Vighi, Rodríguez-Gil, and Daam (2021) |
| Organochloride | Bui Reservoir, Ghana | Contamination of water and sediments with pesticides | Jidauna, Edziyie, and Campion (2020) |

### 2.2 Fungicide effects on aquatic microorganisms and animals

Pesticide pollution of aquatic ecosystems poses severe risks to aquatic microorganisms, macrophytes, invertebrates, and vertebrates (Shefali et al., 2021). de Souza et al. (2020) examined the pesticide monitoring studies from 2012 to 2019 and found the occurrence of pesticides like diuron, dimethoate, and carbendazim in surface waters worldwide. It is well known that carbendazim causes unacceptable effects in aquatic ecosystems (Carazo-Rojas et al., 2018). This azole fungicide acts as an endocrine disruptor, leads to oxidative stress (e.g., accumulation of oxygen reactive species) and immune system disorderliness during larval outreach and fetal stages (Jiang et al., 2015).

As a diverse group of microorganisms, Freshwater fungi have a crucial function in the dynamics of the food cycle (Ittner, Junghans, & Werner, 2018). Due to their ecological importance, fungi are suitable indicators for measuring anthropogenic stresses (Baudy et al., 2020). Field studies show that freshwater fungi are affected by fungicides (Ittner et al., 2018). Zhang, Liu, et al. (2020) found that azoxystrobin at a concentration of 0.2–0.5 $mg\,L^{-1}$ significantly reduced the growth of freshwater eukaryotic algae and *Monoraphidium* sp. McMahon et al. (2012) evaluated the effect of fungicides on biodiversity in freshwater. They found that chlorothalonil in water increases the death of amphibians, gastropods, zooplankton, algae, decreases water transparency, and increases dissolved oxygen. In the study of aquatic microorganism's diversity, it was found that exposure to tebuconazole reduced the production of conidia in an aquatic hyphomycete species (Dimitrov et al., 2014). According to Fernández, Voss, Bundschuh, Zubrod, and Schäfer (2015), in fungal contaminated waters, the structure of microbial communities and fungal biomass changed, and these changes were associated with a 40% reduction in microbial litter decomposition rate. Dijksterhuis, Van Doorn, Samson, and Postma (2011) evaluated the effects of seven fungicides (epoxiconazole, tebuconazole, azoxystrobin, carbendazim, chlorothalonil, fluazinam, and imazalil) on non-target aquatic fungi *Trichoderma hamatum*, *Fusarium sporotrichioides*, *Helicoon richonis* (Ascomycetes), *Cryptococcus flavescens* (Basidiomycetes), *Pythium* spp., and *Mucor hiemalis* (Zygomycetes). These non-target fungi had minimal susceptibility to carbendazim and imazalil in the range of 0.1–25 $mg\,L^{-1}$. Three species of the fungi were susceptible to epoxiconazole because they were sensitive at the lowest test concentration (1 $\mu g\,L^{-1}$). Table 10.3 shows the effects of several chemical fungicides on non-target aquatic animals and microorganisms.

## 3 Reducing the adverse impacts of fungicides

### 3.1 Continuous and regular monitoring of water and soil resources

Three basic strategies could be applied to reduce the adverse effects of pesticides: (i): Improving the system for pesticide residues monitoring in the ecosystems; (ii): Providing the pesticide risk assessment by considering the specific climate and soil conditions of the region; and (iii): Producing pesticides that have the least toxicity (Astaykina et al., 2020). Regular monitoring of natural water resources can be an effective measure to reduce fungicides and other types of pesticides. The Soil Water Assessment Tool (SWAT), as used by Cambien et al. (2020), was investigated to simulate the fate of pendimethalin and fenpropimorph in the en-

**TABLE 10.3** The effect of fungicides on aquatic non-target organisms.

| Fungicide | Effect | Non-target organism | Reference |
|---|---|---|---|
| Tebuconazole | Disorder on the regulation of ion and $Na^+/K^+$-ATPase activity | Fish (*Cirrhinus mrigala*) | Subbiah, Ramesh, Ashokan, and Narayanasamy (2020) |
| Chlorothalonil | Harmful effects on the survival of invertebrates and hyphomycetes | Insect larvae (*Sericostoma pyrenaicum*) and aquatic fungi (Hyphomycetes) | Cornejo et al. (2020) |
| Triticonazole | Animal hypoactivity in 100 μM fungicide treatment | Zebrafish larvae | Souders et al. (2020) |
| Pyraclostrobin, methyl-thiophanate | Hypoactivity and decrease in the total distance traveled, a decrease in non-protein thiol content as well as in catalase activity | Adult Zebrafish | Bevilaqua et al. (2020) |
| Azoxystrobin | Increase in Lipid peroxide and glutathione S-transferase and decrease in glutathione superoxide dismutase in the gastrointestinal tract | Freshwater snail (*Lymnae luteola*) | Ali, Ibrahim, Hussain, and Abdel-Daim (2021) |
| Myclobutanil, cymoxanil, azoxystrobin | Cell viability reduction | Bacteria: (*Rhodopirellula rubra*, *E. coli*, *Pseudomonas putida*, *Arthrobacter* spp.); microalgae: (*Raphidocelis subcapitata*); Macrophytes: (*Lemna minor*) | da Conceição Marinho et al. (2020) |
| Tebuconazole, clotrimazole, terbinafine | Decrease in fungal biomass and reproduction rate reduction, negative effect on microbial decomposers | Aquatic fungal communities | Pimentão, Pascoal, Castro, and Cássio (2020) |
| Prothioconazole | Spawning inhibition, shortening of body length, and formation of pericardial and york cysts | Adult Zebrafish | Tian et al. (2019) |

vironment. SWAT was seen to be an excellent tool for studying the impact of pesticides on water, especially in regions with no data. Input data such as high-resolution soil maps, accurate pesticide application rates, and land management performance are needed to improve the performance of this model. Such mathematical models have advantages that allow us to consider a wide range of soil characteristics and hydrological, climatic, agricultural characteristics, and other parameters. Accordingly, the mathematical models of pesticide behavior in soils, ground and surface water (PEARL 4.4.4, STEP 1–2 SWASH, PERSAM, etc.) are widely used in (Astaykina, Streletskii, Maslov, Kazantseva, et al., 2020).

Therefore, regular and continuous monitoring is recommended to prevent pesticide leaching to groundwater sources. The distance from surface water bodies to sprayed agricultural land can also relieve runoff from agricultural land (Zubrod et al., 2019). Buffer strips, erosion rills, constructed wetlands are effective measures to diminish pesticide concentrations in surface water bodies. Lyu et al. (2018) demonstrated the high fungicide tebuconazole (TEB) removal efficiency in unsaturated constructed wetlands. The pesticide removal efficiency is more significant for pesticides with hydrophobic attributes (low solubility and high KOC) (Vymazal & Březinová, 2015).

## 3.2 Bio-fungicides and reduction of chemical fungicides consumption

Recently, biological fungicides have attracted a lot of attention due to their eco-friendly traits and the alternative of chemical fungicides (Nasran et al., 2020). Biofungicides are antagonistic fungi and bacteria used as an active ingredient to combat fungal diseases (Kharwar, Upadhyay, Dubey, & Raghuwanshi, 2014). Soil microbes interact with each other (Albarracín Orio et al., 2020), and microorganisms' biological control of soil-borne phytopathogens has been accepted as a potential strategy in optimizing crop production. *Trichoderma* sp. is a fungal genus found in many habitats, especially in root and soil ecosystems (Odoh et al., 2020). They can reduce the destructive effects of pathogens (Carro-Huerga et al., 2021). In a study by Kamaruzzaman, Islam, Polash, and Sultana (2021), *Trichoderma asperellum* inhibited the radial growth of *Botrytis cinerea* by 93% and *Sclerotinia sclerotiorum* by 91%. Macena, Kobori, Mascarin, Vida, and Hartman (2020) evaluated the *Trichoderma asperellum* and *T. harzianum* antagonistic activity against six pathogenic fungal isolates of *Sclerotinia sclerotiorum* Thiophanate-methyl fungicide considered as a standard. Laboratory analysis showed that thiophanate-methyl and *Trichoderma* spp. is involved in suppressing carpogenic and myceliogenic germination of Sclerotia. Teixeira et al. (2021) showed that *Bacillus velezensis* had antagonistic activity against *Sclerotinia sclerotiorum*, *Macrophomina phaselonia*, *Botrytis cinerea*, and *Rhizoctonia solani*, and it reduces their mycelial growth by about 60%. Wang et al. (2021) showed that *P. fluorescence* inhibited the conidial germination and mycelium growth of *P. italicum* by 77.86% and 42.14% by producing volatile organic compounds (VOCs). Also, exposure to VOCs produced by *Trichoderma* sp. increased root and leaf number, total chlorophyll content and biomass in lettuce (Wonglom, Ito, & Sunpapao, 2020). When *Bacillus velezensis* was added directly to the soil, the fresh aerial weight of pepper, tomato, cucumber, and pumpkin increased by 63.3%, 53%, 100.8%, and 129.2%, respectively (Torres et al., 2020). Goswami and Deka (2020) found that the *Bacillus altitudinis* strain has significant efficacy in root colonization and strongly inhibits pathogens such as *Colletotrichum gloeosporioides*, *Corynespora cassiicola*, *Fusarium verticillioides*, and *Sclerotinia sclerotiorum* up to 83% in experimental conditions.

Luksiene, Rasiukeviciute, Zudyte, and Uselis (2020) investigated the effect of ZnO nanoparticles as an alternative fungicide on *Botrytis cinerea* in strawberries. They found them to reduce *B. cinerea* radial growth by only 12% in the dark, but photoactivated ZnO nanoparticles reduced the growth of *B. cinerea* up to 80%. Sarker et al. (2020) investigated the effect of Bio-nematicide, BAU-biofungicide, and Bio-nematicide + BAU-biofungicide treatments on root nodules of two varieties of soybeans. In the third treatment, the best plant yield was observed in the shoots and roots, fresh stems and roots, seed weight, and the number of nodes. Besides, the number of galls and adult female nematodes decreased. Table 10.4 summarizes the studies of antifungal microbes. Tiwari, Singh, Trivikram, Singh, and Singh (2020) also evaluated the bio-efficacy of *Bacillus subtilis* as a bio-fungicide on tomato leaf spot disease by using a combination of the chemical fungicide (Copper oxychloride) and the bio-fungicide, Taegro® (containing *Bacillus amyloliuefaciens*, strain FZB24). Results of the study showed that

TABLE 10.4 Some microorganisms with antifungal properties against phytopathogens.

| Strain | Fungal pathogen | Plant | Reference |
|---|---|---|---|
| *Burkholderia gladioli* | *Magnaporthe oryzae*, *Gibberella fujikuroi*, *Sarocladium oryzae*, *Phellinus noxius*, *Colletotrichum fructicola* | Rice | Lin et al. (2021) |
| *Beauveria bassiana* | *Botrytis cinerea* | Tomato, Chili pepper | Barra-Bucarei et al. (2020) |
| *Pseudomonas fluorescens* | *Penicillium italicum* | Postharvest citrus fruits | Wang et al. (2021) |
| *Bacillus megaterium* | *Rhizoctonia solani* | Fab Bean | Hashem et al. (2021) |
| *Bacillus velezensis* | *Alternaria alternata*, *Fusarium oxysporum*, *Monilinia fructicola*, *Magnaporthe oryzae*, *Thanatephorus cucumeris*, *Sclerotinia sclerotiorum* | Tomato, pepper, pumpkin | Torres et al. (2020) |
| *Enterobacter* sp. | *Pestalotiopsis versicolor* | Bayberry | Ahmed et al. (2021) |
| *Trichoderma* sp. | *Corynespora cassiicola*, *Curvularia aeria* | Lettuce | Wonglom et al. (2020) |
| *Paenibacillus* sp. | *Fusarium oxysporum* f.sp. *lycoperi* (FOL) | Tomato | Vinchira-Villarraga, Castellanos, Moreno-Sarmiento, Suarez-Moreno, and Ramos (2021) |

Taegro® could reduce the leaf spots in tomatoes and increase yield and growth compared to control samples. However, the treatment of Taegro®+Copper oxychloride had better results than the treatment of Taegro® alone. Mohamad (2020) used a potential natural fungicide extracted from fruit waste such as pumpkin seeds (*Cucurbit maxima*) and pomegranate peel (*Punica granatum*) to control *Aspergillus niger*. Mixtures of both compounds were seen to be effective against *A. niger*.

Consequently, a mixture of substances extracted from pumpkin seeds and pomegranate peel can be considered a commercial bio-fungicide. Therefore, using rhizosphere soil microbes and detecting bioactive metabolites is essential for the development of fungicides in the future (Shen et al., 2020). Since it is impossible to replace chemical fungicides with bio-fungicides completely, their combined use can reduce the adverse effects of chemical fungicides.

## 3.3 Determination/detection of pesticides residues in water resources

Nowadays, new technologies have emerged to detect pesticides from terrestrial and aquatic ecosystems (Feng et al., 2020). Numerous methods, such as enzyme immunoassay, capillary electrophoresis, liquid and gas chromatography (Samsidar, Siddiquee, & Shaarani, 2018), as well as relatively new methods, such as biosensors (Steffens, Steffens, Marcia Graboski, Manzoli, & Lima Leite, 2017) and differential pulse voltammetry (DPSV), are used to detect pesticides in environmental objects, including water.

Capillary electrophoresis is based on the separation of ions by size and charge in thin capillaries. This method is used to analyze thiabendazole and carbendazim residues in water and characterized by low detection limits (Oliveira, Loureiro, de Jesus, & de Jesus, 2017). Capillary electrophoresis is also used in conjunction with liquid chromatography and mass spectrometry.

The enzyme immunoassay, which is one of several methods, is based on the binding of a pesticide-antigen to a particular antibody, followed by simple photometric, fluorimetric, luminescent, or electrochemical detection. This method, which is cheap and straightforward in application, can be used in the field, and is well suited for water analysis (Morozova, Levashova, & Eremin, 2005). Currently, the method continues to be actively applied for widely-used pesticides, such as glyphosate, neonicotinoids, and organophosphate (Reynoso, Torres, Bettazzi, & Palchetti, 2019). Tebuconazole residues in water were analyzed by enzyme immunoassay and chromatography, and the comparison of the results showed that the enzyme-linked immunosorbent assay was as accurate as chromatography (Chen et al., 2019).

Biosensors are a system that includes a bio-recognition element and a signal conversion method (Verma & Bhardwaj, 2015). Enzyme-based biosensors for pesticide detection are based on the inhibition of various enzymes that depend on the type of pesticide compound (Bucur, Munteanu, Marty, & Vasilescu, 2018). Biosensors based on aldehyde dehydrogenase, tyrosinase, and laccase have shown potential as an easy determination technique for dithiocarbamate fungicides with low solubility in water and other solvents (Fanjul-Bolado et al., 2021).

Modern versions of DPSV use the inorganic part to recognize substances, such as nanoparticles or polymers. For example, this technology has been used to detect clomazone in water using platinum-based multilayer carbon nanotubes (Pt-MWCNT) and voltammetric detection (Milićević et al., 2020). One of the advantages of this method is simplicity and low cost while maintaining the high accuracy of the determination. The disadvantage of sensory methods and enzyme immunoassay is the need to create systems to analyze certain substances, which makes these methods less universal.

The main part of methods used for the quantitative determination of pesticides in water is chromatographic methods, including high-performance liquid chromatography (HPLC) and capillary gas chromatography. The principle of chromatographic methods is to distribute moving components between stationary and mobile phases.

Due to their thermal instability, many pesticides cannot be analyzed by gas chromatography (van der Hoff & van Zoonen, 1999). Ultraviolet and fluorimetric detectors are used in the liquid chromatography of pesticides. Mass spectrometric detection is used in both gas and liquid chromatography. It is currently the most preferred method for pesticide analysis (Chen et al., 2019) due to its universality and high accuracy. Mass spectrometry involves the ionization of compounds and the detection of the abundance of ions. It should be emphasized that any pesticides can be analyzed using chromatography, and the vast majority of work on the determination of pesticides is carried out by chromatographic methods.

## 4 Future perspectives

Chemical fungicides play a significant role in modern agriculture (Skevas, Oude Lansink, & Stefanou, 2013). There can be no doubt that pesticide use may pose risks to the environment and human health [Sustainable Use Directive 2009/128/EC and Plant Protection Products

Regulation (EC) 1107/2009]. But the benefits of fungicides would outweigh the risks if they are implemented following recommended application rates. Non-target effects of synthetic fungicides can be minimized by developments in organic chemistry and distribution systems that reduce the fungicide doses (Thind, 2017). For example, water-soluble bags and carriers for pesticide preparation as starch, chitosan, clay, lignin, sodium alginate, synthetic polymers, and activated carbon (Yang, Zang, Zhang, Wang, & Yang, 2019). These technologies have advantages such as the controlled release of pesticides, lower toxicity, reduced risk of pesticide residues in soil and water, and enhanced product effectiveness to target organisms.

Innovations in biotechnology are the future perspectives of fungicides; ; ; .

New-generation fungicides have a novel mode of action. These plant protection products are very effective even at low application rates, are more target unique, and produce no or minimal residue on harvested crops (Adeniyi, Kunwar, Dongo, Animasaun, & Aravind, 2020).

An integrated approach combining biocontrol agents with synthetic fungicides also reduces pest pressure (Ons et al., 2020; Zhang, Godana, et al., 2020). Bio-fungicides are the focused areas of research in fungicide discovery. However, the recent study shows that bio fungicides have not attained the required application level to displace chemical fungicides. There are several problems in terms of stability, the field uses and distribution systems (Koul, 2019). Use of nanoformulations of Ag, Cu, $SiO_2$, and ZnO, nanobiofungicides, and financing in exploiting the increasingly accessible genome sequences of the most harmful phytopathogenic fungi. The molecular genetic approaches are the powerful alternatives for ecologically sustainable pest management in the nearest future (Abd-Elsalam, Al-Dhabaan, Alghuthaymi, Njobeh, & Almoammar, 2019). Nanotechnology can also help overcome the limitations of common fungicides in plant disease management and contribute to creating a safe ecosystem (Lipsa, Ursu, Ursu, Ulea, & Cazacu, 2020).

In conclusion, the efforts of chemistry, biotechnology and the role of genetic engineering will possibly help to reduce the chances of resistance development and minimize the negative consequences to the environment and human health. These innovations are impossible without efficient and environmentally sound pest control management (Popp, Petö, & Nagy, 2013). Management measures should also be taken to protect the biodiversity of fungi (Irga, Dominici, & Torpy, 2020). Total investment in pest management and new scientific knowledge should be grown to satisfy the food requirements of a growing population and prevent unnecessary damage to the environment.

## 5 Conclusions

As a result of global population growth and the need for maintaining food security, agricultural land use and chemical pesticide consumption have increased. Global pesticide use has doubled from 1990 to 2018. The use of pesticides also is growing due to the emergence of new pests and their resistance. Despite the benefits of pesticides in maintaining crop yield, they have adverse environmental consequences. Pesticides are transmitted to water resources by leaching and surface runoff, which are dangerous to human and environmental health. Fungicides are less studied than other pesticide types, while they have many negative effects on non-target aquatic and terrestrial organisms. Aquatic microorganisms such as microalgae, bacterial and fungal communities are affected by fungicides. These effects included cell viability, biomass, diversity, and abundance reduction. Fungicides also have adverse effects on soil microbial and animal communities.

Genetic damage caused by fungicides can lead to cancer and behavior change in organisms. Management strategies should be used to reduce the adverse effects of fungicides. Regular and continuous monitoring of water and soil resources using tools and models such as SWAT or PEARL 4.4.4 and STEP 1–2 can help fungicide detection in the water and send out hazard warnings. The exploration of antifungal bacterial strains and biocontrol agents (BCAs) can also reduce fungicide consumption. Consequently, nanotechnology, biotechnology, and genetic engineering can be promising in reducing the adverse effects of fungicides and maintaining environmental and human health.

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