Effects of Triazole Fungicides on Soil Microbiota and on the Activities of Enzymes Found in Soil: A Review

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Abstract: Triazole fungicides can manifest toxicity to a wide range of non-target organisms. Within this study we present a systematic review of the effects produced on the soil microbiota and activity of soil enzymes by the following triazole fungicides: cyproconazole, difenoconazole, epoxiconazole, flutriafol, hexaconazole, metconazole, myclobutanil, paclobutrazole, propiconazole, tebuconazole, tetraconazole, triadimenol, triadimefon, and triticonazole. Known effects of the triazole fungicides on the soil activity are dose dependent. High doses of triazole fungicides strongly affects the structure of the microbial communities in soil and usually decrease the soil microbial population and the activities of enzymes found in soil.

Keywords: triazole fungicides; soil microorganisms; soil enzymes

1. Introduction

Pesticides are heterogeneous chemicals used widely in agriculture. Their design as bioactive molecules to exterminate different animal, vegetal, or fungal species implies that they are toxic by definition [1]. Pesticides are used mostly on agricultural land and private gardens, but also some industries utilize pesticides to clear roadways of weeds and shrubs, to kill invasive plants, or to control algae growth in bodies of water [1]. The use of pesticides for crop protection is expected to increase based on a growing world population and the need for more food supplies. In 2014 the worldwide consumption of pesticides was about two million tons per year: 45% in Europe, 25% in the USA, and 30% in the rest of the world [2]. Worldwide, 40% of pesticide use is contributed to herbicides, 17% to insecticides, and 10% to fungicides. The group “fungicides and bactericides” was the most sold group of pesticides in the EU in 2019 [3]. There are more than 1000 active ingredients in various types of pesticides used worldwide [4]. The formulation of new pesticides is growing due to the appearance of resistant pests, growing global population and the regulation of pesticides. It is considered that more than 98% of sprayed pesticides reach a destination other than their target species, becoming pollutants of air, water, and soil [5]. Accidental exposures occur to non-target organisms in the areas where they are applied. Part of the applied pesticides persist in the soil, while other parts are lost via vaporization or leaching to the groundwater following rainfall or irrigation [6].

Soil health (also referred as soil quality) is defined as “the capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and promote plant and animal health” [7]. The properties that assure the soil health are dependent on the type of soil, soil microorganism diversity, and the activity of soil [8]. The concept of soil health is directly related to the growth of plants, as the soils with appropriate properties
support abundant plant growth and are able to withstand the variability of environmental conditions. Furthermore, the role of soil microorganisms in maintaining the soil fertility and productivity through biological processes is considered a key strategy toward agricultural sustainability [9].

Soil enzymes are considered to originate predominantly from microorganisms, but also from residues of plants or animals. They accumulate in the soil either as free enzymes or stabilized mainly on soil organic matter, these being the most active part of soil organic components [8]. Soil enzymes participate in all biochemical processes taking place in soil and that are necessary for microbial life functions by increasing the reaction rate of organic matter decomposition and releasing nutrients into the soil environment. Because of their stability and sensitivity, soil enzymes are used as indicators of soil health [10].

Ecosystem impacts of pesticides are numerous and include soil microorganism response and effects on the activities of enzymes found in soil [11]. These effects depend on both the physicochemical properties of the pesticide (especially molecular weight, lipophilicity) and the properties of the soil (texture, structure, pH, adsorption capacity, biological activity, oxygen content, temperature, and moisture, etc.) [4].

This study focuses on the effects of triazole fungicides on the soil microorganisms and on the activities of enzymes found in soil, as microorganisms and enzymes are very sensitive to stress and respond to contamination faster than other parameters [11]. Literature data show that the fungicide residues may accumulate in the soil and cause changes in the soil physicochemical properties (pH, organic matter content, content of NH$_4$-N, NO$_3$-N and phosphate, etc.) [12]. These effects are not considered in the present study. Triazole fungicides are the basis of disease management strategies worldwide being used as seed treatments or foliar sprays onto the growing crops such as cereal crops, market gardening, ornamental cultures, and vineyards [13]. Their wide use is due to the very broad spectrum of effectiveness against the main diseases of these crops and it is forecasted to intensify. In the European Union, (EU) fungicide sales accounted for more than 40% of the total pesticide sales in 2019 [14] (Figure 1).

![Figure 1](http://appsso.eurostat.ec.europa.eu/nui/submitViewTableAction.do, accessed on 8 April 2021).

Triazole fungicides are inhibitors of the enzyme lanosterol 14α-demethylase, which is essential for the biosynthesis of ergosterol, a key fungal cell membrane component, thus inhibiting fungal growth [15]. Data in the literature illustrate the environmental effects of the pesticides, but the effects of the triazole fungicides have received less attention. For instance, a simple search in the “Environment” section in the SpringerLink online collection of scientific publications in April 2021 led to results illustrated in Figure 2. The lower number of published papers regards these fungicides.
Agriculture
Agriculture

Difenoconazole
Cyproconazole
Tetraconazole 1-[2-(2,4-dichlorophenyl)-3-(1,1,2,2-tetrafluoroethoxy)propyl]-
Paclobutrazol
Myclobutanil
Triticonazole
Metconazole
Triadimefon
Triadimenol

bonds count, TPSA—topological polar surface area, DT 50 and DT90 the periods after that 50% and respectively 90% of the,

1-(4-chlorophenoxy)-3,3-dimethyl-1-(1,2,4-triazol-1-yl)butan-2-, 893
1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)pentan-3-, x FOR PEER REVIEW 3 of 18
1-[3-(2-chlorophenyl)-2-(4-fluorophenyl)oxiran-2-yl]methyl]-
2-(2,4-dichlorophenyl)-1-(1,2,4-triazol-1-yl)hexan-2-ol 314.2 3.7 1 3 6 50.9 49–200
(persistent) 329–803
1-(2-fluorophenyl)-1-(4-
1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-ylme-
1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-ylmethyl)cyclopentan-1-ol 317.8 3.1 1 3 3 50.9 36.1–242
1-(2-fluorophenyl)-1-(4-
1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-ylme-
1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-ylmethyl)cyclopentan-1-ol 319.8 3.7 1 3 4 50.9 26.6–368.5
1-(2-fluorophenyl)-1-(4-
1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-ylme-
1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-ylmethyl)cyclopentan-1-ol 322.3 4.1 0 5 5 49.2 76.3–423.9
1,2,4-triazole 329.8 3.2 0 4 4 43.2 0.75–247.8
1,2,4-triazole 342.2 3.5 0 4 5 49.2
1,2,4-triazole 372.14 4.4 0 7 7 39.9

The position of the nitrogen atoms in the 1,2,4-triazole compounds.

Figure 2. Number of scientific publications regarding the environmental effects of various pesticides found in the “Environment” section in the SpringerLink online collection in April 2021 (https://link.springer.com/; SCOPUS, https://www.elsevier.com/solutions/scopus) and collections for all years until April 2021. For every fungicide, the data are presented chronologically.

2. Properties of Triazole Fungicides

All the fungicides considered in this study belong to the class of 1,2,4-triazole compounds (Figure 3).

Figure 3. The position of the nitrogen atoms in the 1,2,4-triazole compounds.
The molecular structure and physicochemical properties of the fungicides greatly determine their degrees of interaction with the environment. Furthermore, these properties, together with the properties of the soil, are very important for the fungicide’s action and are linked to their mobility and persistence in the soil [19]. They also influence dissociation in water, bioaccumulation, durability in the environment, and determine the effects on the target and no-target organisms [19]. An increased potential for contamination of soil is found for the molecules that are more persistent and mobile [20]. The physicochemical properties of the fungicides under investigation are presented in Table 1, together with their rates of degradation, i.e., the period after which 50% (half-life, DT$_{50}$) and 90% (DT$_{90}$) of the fungicide has been degraded. These data are extracted from PubChem database [21] and Pesticide Properties Data Base [22], respectively.

### Table 1. IUPAC names, physicochemical and degradation rates of investigated triazole fungicides: MW—molecular weight, logP—partition coefficient, HBD—hydrogen bonds donors, HBA—hydrogen bonds acceptors, RBC—rotatable bonds count, TPSA—topological polar surface area, DT$_{50}$ and DT$_{90}$ the periods after that 50% and respectively 90% of the fungicide to be degraded.

| Fungicide Common Name | IUPAC Name | MW (g/mol) | logP | HBD | HBA | RBC | TPSA (Å$^2$) | DT$_{50}$ for Field Studies (Days) | DT$_{90}$ for Field Studies (Days) |
|-----------------------|------------|------------|------|-----|-----|-----|------------|----------------------------------|----------------------------------|
| Cyproconazole         | 2-(4-chlorophenyl)-3-cyclopropyl-1-(1,2,4-triazol-1-yl)butan-2-ol | 291.77 | 2.9  | 1   | 3   | 5   | 50.9       | 62.1–501.2 (persistent)          | 179–1000                         |
| Difenoconazole        | 1-[[2-[2-chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-yl][methyl]-1,2,4-triazole | 406.3  | 4.0  | 0   | 5   | 5   | 58.4       | 20–265 (persistent)              | 68–879                           |
| Epoxiconazole         | 1-[[3-(2-chlorophenyl)-2-(4-fluorophenoxy)oxiran-2-yl][methyl]-1,2,4-triazole | 329.8  | 3.2  | 0   | 4   | 4   | 43.2       | 0.75–247.8 (persistent)          | 183.7–10.000                     |
| Flutriafol            | 1-(2-fluorophenyl)-1-(4-fluorophenyl)-2-(1,2,4-triazol-1-yl)ethanol | 301.29 | 2.3  | 1   | 5   | 4   | 50.9       | 316–4089 (very persistent)       | 1051–13,583                      |
| Hexaconazole          | 2-(2,4-dichlorophenyl)-1-(1,2,4-triazol-1-yl)hexan-2-ol | 314.2  | 3.7  | 1   | 3   | 6   | 50.9       | 49–200 (persistent)              | NA                               |
| Metconazole           | 5-[4-(chlorophenyl)methyl]-2,2-dimethyl-1-(1,2,4-triazol-1-ylnmethycyclopentan-1-ol | 319.8  | 3.7  | 1   | 3   | 4   | 50.9       | 26.6–368.5 (persistent)          | 102.9–1000                       |
| Myclobutanil          | 2-(4-chlorophenyl)-2-(1,2,4-triazol-1-ylnmethylenanenitrile | 288.77 | 2.9  | 0   | 3   | 6   | 54.5       | 9–58 (Moderately persistent)     | 637–1906                         |
| Paclobutrazol         | 1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)pentan-3-ol | 293.79 | 3.2  | 1   | 3   | 5   | 50.9       | 27.2–60.8 (persistent)           | 46.7–202                         |
| Propiconazole         | 1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl][methyl]-1,2,4-triazole | 342.2  | 3.5  | 0   | 4   | 5   | 49.2       | 15.3–96.3 (moderately persistent) | 108–525                          |
| Tebuconazole          | 1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-yl)pentan-3-ol | 307.82 | 3.7  | 1   | 3   | 6   | 50.9       | 25.8–91.6 (moderately persistent) | 66–304                           |
| Tetraconazole         | 1-[[2-(2,4-dichlorophenyl)-3-(1,1,2,2-tetrafluoroethoxy)propyl]-1,2,4-triazole | 372.14 | 4.4  | 0   | 7   | 7   | 39.9       | 136–1688 (moderately persistent) | 453–5606                         |
| Triadimenol           | 1-(4-chlorophenoxy)-3,3-dimethyl-1-(1,2,4-triazol-1-yl)butan-2-ol | 295.76 | 3.1  | 1   | 4   | 5   | 60.2       | 24.1–83.7 (persistent)           | 76.3–423.9                       |
| Triazinefom           | 1-(4-chlorophenoxy)-3,3-dimethyl-1-(1,2,4-triazol-1-yl)butan-2-one | 293.75 | 2.8  | 0   | 4   | 5   | 57         | 26 (non-persistent)              | NA                               |
| Tritionoxan           | (5E)-5-[4-(chlorophenyl)methyliden]-2,2-dimethyl-1-(1,2,4-triazol-1-ylnmethycyclopentan-1-ol | 317.8  | 3.1  | 1   | 3   | 3   | 50.9       | 36.1–242 (persistent)            | 329–803                          |

Data presented in Table 1 illustrate that triazole fungicides are moderately lipophilic (the median logP value is 3.35), moderately flexible (the median value of the rotatable bonds is 5) and their hydrogen bonding capacity is quite low. All these properties are important for both their efficiency as fungicides as well as for their effects on the soil.
microorganisms and on the activity of enzymes found in the soil. Persistence of triazole fungicides in soil may be attributed to their lower mobility and higher sorption into soil due to the hydrophobic nature of the fungicides and their low molecular weight. Due to the persistence of these fungicides in soil, it becomes important to investigate their effect on the soil microbial growth, survival, and activity.

3. Effects of Triazole Fungicides on Soil Microorganisms

A good biomarker of changes in soil functioning is represented by its structure of microbial communities as they take part in various interactions between organisms and biological processes. This structure of microbial communities is determined by the soil properties, but is also influenced by the use of fertilizers and/or pesticides [23]. We review here the effects produced by the triazole fungicides used for protecting crops on the soil microorganisms. We could not identify information regarding the effects of cyproconazole, metconazole and triadimenol on the soil microorganisms. Cyproconazole is considered to not really be biodegradable, [24] and it illustrates low toxicity to microbes in sewage sludge [25]. The registration decision of metconazole in Canada in 2015 revealed that, when used according to the label directions, this fungicide does not pose an unacceptable risk to the environment [26]. The effects of triazole fungicides on soil microorganisms are found in Table 2.

**Table 2.** Effects of high doses of triazole fungicides on soil microorganisms: red cells indicate decreased values compared to the control, blue cells indicate increased values compared to the control, green cells reveal no effects of the fungicides on the soil microbiota, yellow cells illustrate modifications in the structure of microbial communities, white cells correspond to the lack of available data.

| Triazole Fungicide | Soil Microbial Activity | Microbial Biomass | Total Microbial Population | Population of Fungi | Population of Bacteria | Structure of the Microbial Communities |
|--------------------|-------------------------|-------------------|---------------------------|---------------------|------------------------|---------------------------------------|
| Difenoconazole     |                         |                   |                           |                     |                        |                                       |
| Epoxiconazole      |                         |                   |                           |                     |                        |                                       |
| Flutriafol         |                         |                   |                           |                     |                        |                                       |
| Hexaconazole       |                         |                   |                           |                     |                        |                                       |
| Myclobutanil       |                         |                   |                           |                     |                        |                                       |
| Paclobutrazole     |                         |                   |                           |                     |                        |                                       |
| Propiconazole      |                         |                   |                           |                     |                        |                                       |
| Tebuconazole       |                         |                   |                           |                     |                        |                                       |
| Tetraconazole      |                         |                   |                           |                     |                        |                                       |
| Triadimefon        |                         |                   |                           |                     |                        |                                       |
| Triticonazole      |                         |                   |                           |                     |                        |                                       |

3.1. Difenoconazole

The effect of difenoconazole on the soil microorganisms was studied in a clay-loam soil [27]. The fungicide was applied in concentrations of 5, 50 and 500 mg/kg of soil and the samples were incubated under laboratory conditions for three months. The microbial parameters were registered at days 7, 30, 60 and 90 [27]. The difenoconazole in the concentration of 5 mg/kg of soil did not cause significant changes in the soil microbial parameters, but in the concentration of 500 mg/kg of soil, the difenoconazole caused a decrease in the microbial activity in the soil [27]. Another study considered a loamy-sand soil that was treated with difenoconazole in the recommended dose (0.04 mg/kg of soil) and maintained in laboratory conditions [28]. The microbial communities were assessed at 0, 7, 14, 28, 56 and 84 days after soil contamination. The effect of difenoconazole on the population of microorganisms was acute; the microbial biomass was reduced as the microorganisms spent more energy in the detoxification processes than in microbial growth [28].
3.2. Epoxiconazole

The effect of epoxiconazole was studied on a sandy loam soil [29]. The epoxiconazole was applied in concentrations of 0.25 mg/kg and 25 mg/kg of soil, corresponding to two and 20 times the field rate, respectively. The soil samples were incubated for 28 days. The soil fungal ergosterol content was decreased by about 30% after seven days of incubation in the soil samples treated with epoxiconazole, and there were no differences between the two concentrations applied [29]. This illustrates that a particular fraction of the soil fungal population was affected by the fungicide and the lower concentration of the fungicide was sufficient to inhibit this fraction. After 14 days of incubation, the soil fungal ergosterol content and the soil microbial biomass in samples treated with epoxiconazole at both concentrations were similar to those of the control soil [29]. Furthermore, the soil microbial biomass had not been affected by the application of epoxiconazole, this parameter being identical for the 28 days of incubation. It indicated that the soil ergosterol content was a more sensitive parameter when characterizing the effect of epoxiconazole than was the soil microbial biomass [29].

3.3. Flutriafol

The effect of flutriafol on the soil microorganisms was tested using brown soil cultivated with corn [30]. The cellulose decomposition rate was used to measure the effect of flutriafol on microbial cellulolytic activity in soil samples. Four concentrations of flutriafol were considered (0.17, 1.7, 17 and respectively 170 mg/kg of soil), corresponding to approximately 1, 10, 100 and 1000 fold the normal field rate. The incubation time was 50 days [30]. At low concentrations of the fungicide (0.17 and 1.7 mg/kg), no significant differences were observed for the first 15 days of incubation in comparison to the untreated soil. Cellulolytic fungal activities were inhibited by high doses of flutriafol (17 and 170 mg/kg) in the first 15 days of incubation and the adverse effects disappeared after 15 days [30]. Changes in the soil microflora resulted in the decrease of the fungal population in favor of bacteria [30].

3.4. Hexaconazole

A study by Kalam and Mukherjee (2001) [31] considered soil from a rice-field and hexaconazole concentrations of 0.5, 1, 2.5, 5, 10 mg/kg of soil, and examined the effects of the fungicide on the total microbial population and on various species of bacteria. In this case, the soil samples were incubated up to 35 days at 28°C. The total microbial population decreased in the first 21 days after application, regardless the dose of the fungicide, up to 61% for the concentration of 10 mg/kg of soil. Thereafter, the fungicide degraded, and this was accompanied by an increase in the population of actinomycetes. Consequently, the hexaconazole had some inhibitory effects on the microflora from the soil, and this may have affected the soil fertility [31]. The effect of the hexaconazole on the soil microorganisms was also studied using red and black paddy soils from China. The fungicide was applied in two different concentrations: the field rate, T1 (0.6 mg/kg of soil) and 10 times higher than the field rate, T10 (6 mg/kg of soil). The changes in the communities of soil microorganisms were analyzed during 90 days of incubation [32]. The hexaconazole was rapidly degraded initially, and this phase was followed by further slow-decline phase degradation; the half-lives of the two doses of pesticide ranged from 270 to 845 days in the red soil and from 122 to 135 days in the black soil. The initial rapid degradation of hexaconazole may be due to the metabolism of the fungicide by microorganisms such as to reduce the poison in order to survive, or to use it as a carbon source for growth. The second phase, which was characterized by the slow degradation, may have been due to the reduction in total microbial biomass. This study found that the hexaconazole decreased the populations of total bacteria in both types of, which is consistent with the results of other study [33], revealing that the hexaconazole impacted bacteria involved in nitrogen cycling. The bacterial populations were significantly affected in both soil types by the hexaconazole dose and incubation time; in the black soil the decrease in the population of bacteria appeared from day 15 to day 60, and in the red soil
this inhibition appeared in the first 15 days. The outcomes of this study found that the use of the hexaconazole led to toxicity for soil microorganisms with direct consequences on the soil quality [32].

3.5. Myclobutanil

Myclobutanil was applied to tea orchard soil at doses of 0.1, 1, and 10 mg/kg of soil and incubated for 10 days [34]. Sampling was carried out after 1, 3, 5, and 10 days of incubation. This study found a decrease in the soil’s microbial biomass with the increasing of the concentration of myclobutanil and the incubation time [34].

3.6. Paclobutrazol

An assay of the paclobutrazol’s effect on the soil microorganisms was done on sandy loam soil from mango orchards by applying 8 mg/kg of soil in field conditions. The study found the reduction by 58%, 28%, and 28% for total viable counts of bacteria, actinomycetes and fungi, respectively [35]. When the paclobutrazol was applied on soil samples under greenhouse conditions in concentrations of 80 and 160 mg/kg of soil, there was no effect on the soil microorganisms [35]. Another assay considered mung bean plants that were treated with paclobutrazol and cultivated for three seasons in order to determine the effect of paclobutrazol on soil microorganisms. This assay demonstrated that the presence of the paclobutrazol negatively affected the bacterial community, especially in the first season [36]. There was another study performed on sandy-loam soil from a mango garden revealing that the community structure of soil bacteria was reduced by the presence of the paclobutrazol. With the paclobutrazol, the amounts of proteobacteria and plantactomycetes were significantly augmented and those of actinobacteria and futilicates were significantly reduced [37].

3.7. Propiconazole

The impact of the propiconazole on soil bacterial populations was investigated using sandy clay loam soil from a strawberry field [38]. The fungicide was applied in two concentrations (10 mg/kg and 100 mg/kg of soil) in both sterile and non-sterile soil samples with two water contents (20.2% and 26.0%), and incubated for 75 days. The dissipation rates of the fungicide were similar for the non-sterile soil with the two water content and after 75 days of incubation there was about 60% of propiconazole remaining in the soil treated with the fungicide concentration of 10 mg/kg and about 80% of propiconazole remaining in the soil treated with the fungicide in the concentration of 100 mg/kg [38]. The dissipation rates of the propiconazole in the concentration of 100 mg/kg applied in sterile and non-sterile soils were similar, and this showed that soil microbes were inhibited by the fungicide when applied in a high dose. The soil microbial communities were disturbed immediately after application of propiconazole, but they recovered after 60 days [38]. Furthermore, changes in the bacterial communities were assessed at 1, 15, 30, 45, 60 and 75 days, respectively [38]. The propiconazole produced changes in the soil bacterial communities, and the composition of microbial communities was not recovered after 75 days [38]. In another study, the effect on the communities of microorganisms of the propiconazole applied in concentrations of 12.5, 25, 50, 100, 200, 400 and 800 mg/kg of soil was assessed for sandy loam soil during 40 days of incubation [39]. The propiconazole inhibited the growths of the fungal cells, and the effect was significantly increased for a concentration of propiconazole higher than 50 mg/kg of soil, while 75% from fungal cells were inhibited at the highest dose (800 mg/kg of soil). The bacterial growth was significantly inhibited by propiconazole for concentrations of at least 200 mg/kg in the first week after application. High levels of propiconazole stimulated bacterial growth after seven and 40 days. It showed the complexity of bacterial growth’s response to propiconazole application as a result of a combination of direct toxicity on some bacteria, adaptation of the bacterial community and elimination of the fungal community [39]. The effect of the propiconazole on microorganism communities was also assessed on other two
types of soils: red sandy loam and deep black soils of paddy rice (Oryza sativa L.) [40]. Different concentrations of propiconazole (1.0, 5.0, 10.0, 15.0 and 20.0 kg/ha) have been considered, and the soil samples were incubated at room temperature for four weeks. At one week of incubation, lower application doses of 1.0 and 5.0 kg/ha increased the growth of bacterial and fungal communities when compared with untreated soil. After long incubation periods (two–four weeks), the population of soil microbes was decreased indicating that the extended application of the fungicide would suppress the soil microbes and affect the soil quality [40]. The decrease in the populations of soil microbes may be due not only to the effect of the fungicide and its persistence in the soil ecosystem, but also to the competitiveness of microbial population for food to fulfil the carbon requirement. Furthermore, the carbon content of the soil is also dependent on the properties of the soil, especially on its content in organic matter [41].

3.8. Tebuconazole

Strickland et al. (2004) [42] performed a 63-day laboratory incubation to evaluate the dissipation of the tebuconazole and its effects on soil microbial activity in a loamy sand soil. Sampling was performed at 7, 14, 21, 28, 42 and 63 days, respectively. This study found that tebuconazole does not have a significant effect on soil microbial biomass when applied in the field rate dose. In another study, the tebuconazole at dosages of 2.7 (the maximum predicted environmental concentration in field conditions), 13.5 and 270 mg/kg soil was used on sandy-loam soil samples that had not been used for agricultural purposes for several years before and had not received any pesticide or fertilizer applications in the three preceding years [15]. The microbial biomass has been assessed at 1, 7, 14 and 28 days. The results of this study showed that the tebuconazole seemed to affect soil microorganisms to a little extent, the adverse impact was only observed for the highest concentration of tebuconazole and shortly after the application. It also underlines that the microorganisms could use the fungicide as a substrate that allowed them to survive in amended soil. Fereira and coauthors (2009) [43] considered three types of soils treated with a dose of 0.2 kg/ha of tebuconazole and observed short term inhibitory effects within the first month after treatment; these were recovered after two months in accordance with pesticide dissipation, the recovery process being dependent on the type of soil. The effect of the tebuconazole on soil microorganisms was also studied on a clay-sandy soil by applying the fungicide at concentrations of 5, 50 and 500 mg/kg of soil [44]. Soil sampling was performed at 0, 7, 30, 60 and 90 days of incubation. The tebuconazole degradation revealed an initial rapid phase (30 days) followed by a slow-decline phase. The degradation rates were dependent on the fungicide dose: the higher the tebuconazole concentration, the longer the half-life: 9, 74 and 263 days for 5, 50 and 500 mg/kg of soil, respectively. After 90 days of incubation, 25%, 47% and 59% of the initial tebuconazole concentration still remained in the soil for 5, 50 and 500 mg/kg of soil, respectively [44]. In the tebuconazole-treated soils, the values and activity of microbial biomass were lower when compared to the controls, and at day 30, values of the soil microbial biomass treated with the highest dose of tebuconazole were 94.6% lower than in control [44]. The Environmental Food Safety Authority (EFSA), which is the regulatory authority in charge of the authorization of the active substances of pesticides for all EU countries, suggests acceptable low level effects on soil microorganisms (<25%) for tebuconazole [45]. A three-month laboratory experiment was performed on fluvo-aquic soil samples treated with tebuconazole in concentrations 1, 10, and 100 mg/kg of soil. The microbial parameters have been determined at 0, 7, 30, 60, and 90 days of incubation. A higher concentration of the fungicide had a negative effect on the population of soil fungi, especially in the first 30 days of incubation. This study also found that the effect of tebuconazole on the soil microbiota was dependent on concentration and on the incubation time [46]. Storck et al. (2018) [47] used doses of $1 \times$, $2 \times$, or $10 \times$ the recommended dose (0.6 mg/kg soil) of tebuconazole sprayed on loamy sand soil samples maintained in laboratory conditions and also on soil samples maintained in field conditions. The effect of the tebuconazole on the diversity and com-
position of the soil bacterial community was assessed after 14, 35, 70, and 105 days after treatment. Significant differences were found in the operational taxonomic units in the field experiment between fungicide treatment and control after 70 days of exposure [47]. The tebuconazole induced minor but significant changes in the composition of the soil bacterial community. The bacterial diversity and composition varied over time, especially in the soil samples maintained in laboratory conditions [47]. Another study considered the effects of the tebuconazole applied in concentrations of 0.042, 0.083, 0.125, 1.249, and 2.499 mg/kg of loamy sand soil for 60 days of incubation [48]. Sampling was performed at 20, 40, and 60 days after treatment. Study outcomes demonstrated that the tebuconazole caused significant changes in the proliferation of microorganisms and on their biodiversity, especially at its highest dose [48]. The tebuconazole inhibited the increase of the actinobacteria and the fungi; the decrease in the actinobacteria population ranged from 5.71% (dose 1.249 mg/kg) to 37.25% (dose 2.499 mg/kg), and that of the fungi population from 2.12% (dose 0.125 mg/kg) to 36.81% (dose 2.499 mg/kg) compared to the control samples [48]. This study also found that the adverse effect of this fungicide on soil microorganisms may be decreased by using biostimulating substances such as compost [48]. The effect of the tebuconazole on the microbial population from a sandy loam soil was also evaluated after foliar application of the tebuconazole by spraying it on the leaves of spring barley in the doses of 0.046, 0.093 (the field rate), 0.139, 1.395, and 2.790 mg/plant. The experiment was carried out in a greenhouse and microbiological analyses were performed on day 40 and 60 after treatment. The obtained results revealed that the tebuconazole application had consequences on the soil microorganism populations [49]. The bacterial community was different both in terms of structure and percentage contribution when comparing the soil exposed to the fungicide from the control soil. The proteobacteria prevailed in both the soil treated with the fungicide and in the control soil and it illustrated their capability to colonize both the natural and soil-containing fungicides [49]. The *Bacillus arabhattai*, *B. soli*, and *B. simplex* bacteria have high sensitivity to tebuconazole as their populations decrease compared to the non-contaminated soil in a dose responsive manner. Other species, such as *Ramlibacter tataounensis*, *Azospirillum palatum*, and *Kaistobacter terrae* were found exclusively in the soil contaminated with the fungicide [49]. There was a reduction in the population of fungi in the soil, especially for the highest doses of tebuconazole [49]. Overall, most of the studies reported moderate (sometimes temporary) toxic effects of the tebuconazole to the soil microbial biomass and diversity, the toxicity being dose dependent.

3.9. Tetraconazole

Zhang et al. [50] performed a study regarding the effects of application of tetraconazole on a silty loam soil. The tetraconazole was applied in three concentrations: the recommended field rate (T1, 0.33 mg/kg of soil), three times (T3, 1.00 mg/kg of soil), and ten times higher than the recommended field rate (T10, 3.33 mg/kg of soil) and the effects were registered after 7, 15, 30, 45, 60 and 90 days of incubation [50]. The degradation half-lives of tetraconazole in the silty loam soil were dependent on the applied concentrations and were 69 (T1), 77 (T3) and 87 (T10) days. The application of the tetraconazole decreased the microbial biomass and the activity of soil over the entire incubation period, but for the higher concentrations (T3 and T10), the negative effects on the soil microbiota were not recovered during the 90 days of incubation. The structure of the microbial communities in soil treated with tetraconazole were also affected. The amount of total fungi decreased in the first 30 days and increased up to day 90, probably because the microbial death was followed by the growth of the populations of organisms using the killed biomass as a carbon source. Moreover, the tetraconazole affected non-target bacterial communities, the gram negative being more affected than the gram positive bacteria probably due to the cell walls of the gram positive bacteria that are more resistant on the actions of pesticides [50]. Similar effects of the use of tetraconazole in two concentrations (T1 and T10) on silty loam soils from orchard (and with long history of triazole application) and from grassland (with no known history of fungicide usage) have been found in a laboratory.
investigation of 28 days [51]. Both concentrations of tetraconazole affected the structure and genetic diversity of the bacterial community during the 28 days [51]. These effects were not observed immediately after application of the fungicide, but were on day 28. The dosage of the tetraconazole was the main factor responsible for its effects on the genetic diversity of the bacterial communities [51]. Analysis of the values of the functional biodiversity index revealed that application of the tetraconazole affected metabolic potential of the bacterial community [51]. The growth of the gram positive bacteria in orchard soil was significantly inhibited when the tetraconazole was applied in both T1 and T10 doses. The tetraconazole application led to the increase in the number of tetraconazole-resistant fungi in both types of soil, although none of these soils had previously been treated with tetraconazole. The increase was more pronounced in the orchard soil. Furthermore, after tetraconazole treatment, the microbial activity was lower in the orchard in comparison with the grassland soil [51]. The different response of the microbial communities to application of the tetraconazole may be explained as being related with the physicochemical properties of the soil, the pH and the organic matter content being the more important parameters affecting the response of the bacterial communities to application of the fungicide. It underlines that the type and management of agricultural soil are important factors when assessing the impact of pesticides [51]. The history of soil also influenced the structure of the bacterial community; the tests on the orchard soil found a negative response of the content of phospholipid fatty acid, an indicator of the living microbial biomass, to the application of the fungicide [52].

3.10. Triadimefon

The effect of the triadimefon was studied on a sandy loam soil where the fungicide was applied in concentrations of 0.125 mg/kg (the field rate) and 1.25 mg/kg of soil. The soil samples were incubated for 7, 14 and 28 days, respectively [29]. The soil fungal ergosterol content was reduced by about 30% after 7 days of incubation in the soil samples treated with triadimefon with no significant differences between the two concentrations applied. It indicates that a particular fraction of the soil fungal population seems to be affected by the triadimefon, the lower concentration of the fungicide being enough to inhibit this fraction [29]. After 14 days of incubation, the soil fungal ergosterol content was similar with those of the control soil. The soil microbial biomass registered an inhibition of about 12% after 13 days of incubation, illustrating the side-effects of the triadimefon on the bacterial population of the soil [29]. The impact of triadimefon on the community of the soil microorganisms was also investigated in a study considering both sterile and non-sterile sandy clay loam soils from a strawberry field. The triadimefon was applied in concentrations of 10 mg/kg and 100 mg/kg on soil samples with two water contents (20.2% and 26.0%) and incubated for 60 days. The fungicide dissipated very fast in non-sterile soil compared to sterile soil, more than 50% of triadimefon either in 10 mg/kg or 100 mg/kg concentration was dissipated. There was not a significant effect of the soil water contents on the dissipation of the fungicide. It illustrated that the microorganisms played an important role in the triadimefon dissipation [29]. This study also found that, regardless of the concentration of the fungicide, the soil microbial communities were disturbed immediately after application of the triadimefon and did not recover after 30 days [38]. The bacterial population increased during the first 20 days of incubation with a maximum at day 10 indicating that the triadimenol, the primary metabolite of the triadimefon, may be used as a carbon source. After 20 days of incubation, the bacterial population decreased for both concentrations, the decrease being more pronounced for the concentration of 100 mg/kg. The triadimefon produced changes in the soil bacterial communities. These changes were not recovered after 60 days [38].

3.11. Triticonazole

The triticonazole was applied as a barley seeds dressing in concentrations of 150 and 200 mL/100 kg of grains. The seeds were sown on loamy sand soil and the sam-
pling took place in correlation with the developmental phases of spring barley: emergency (10–13 days), tillering (21–25 days), flowering (51–69 days), and after harvest (71–82 days) [53]. The triticonazole was found to stimulate the bacteria proliferation in the soil and to reduce the population of fungi [53]. It may be due to the fact that bacteria use the fungicide as an additional source of nutrients.

A summary of the effects produced by high doses of triazole fungicides on the soil microbiota is presented in Table 2.

High doses of triazole fungicides strongly affect the microbial populations and activities in almost all types of investigated soil. The mechanisms of action of the triazole fungicides is based on causing membrane dysfunction by inhibiting sterol biosynthesis in fungal cells [11]. The bacterial membranes do not contain sterols, and it leads to the conclusion that the triazole fungicides have an indirect effect on the soil bacterial population [53]. This indirect effect may be explained by the reduction in the microbial biomass and the functional or nutritional connection of bacteria with fungi [54].

4. Effects of the Triazole Fungicides on Enzyme Activities

The enzymatic activity of soil is correlated with the changes proceeding in biogeochemical cycles and in the dynamics of organic matter breakdown. Consequently, the response of enzymes to the stress factors is fast, and it allows prompt evaluation of the extent of ongoing alterations [55]. The assessment of the effects of the pesticides on the enzymes found in soil is usually based on measurements of the activity of several enzymes in the presence of the pesticides dehydrogenase (DHA), urease (UA), phosphatase (PHA) and protease (PA) [9]. We review here the effects produced by triazole fungicides used for protecting crops on the activities of various enzymes found in soil. We were not able to identify information regarding the effects of the cyproconazole, epoxiconazole, flutriafol, metconazole, tetraconazole and triadimenol on the activity of enzymes found in the soil.

4.1. Difenoconazole

The difenoconazole was applied in doses of 5, 50 and 500 mg/kg of a clay-loam soil, the samples were incubated for 7, 30, 60 and 90 days respectively before the DHA activity was assessed. For the doses of 5 and 50 mg/kg, the difenoconazole had no clear effect on DHA activity, but for the dose of 500 mg/kg of soil, an average decrease of 53.6% of the DHA activity was found for all incubation times [27]. Another study considered the difenoconazole applied in doses of 37 mg/kg, 75 mg/kg (the field rate) and 150 mg/kg of chernozem soil samples incubated for 21 days in both field and laboratory conditions (at a constant temperature of 30 °C) for assessing the influence of the pesticide on the activities of dehydrogenase, urease, protease, and acid phosphatase [56]. Among these enzymes, dehydrogenase was the most sensitive to the use of the difenoconazole. When the fungicide was applied in a dose of 0.150 mg/g of soil, there was a 90.16% decrease of the DHA activity after 21 days in controlled laboratory conditions. Similarly, difenoconazole in the highest concentration led to the decrease in UA activity of 33.84% in field conditions and 29.63% in laboratory conditions. With respect to the acid phosphatase, there was a decrease of 49.73% of the PHA activity for the highest dose of difenoconazole applied to the soil samples maintained in laboratory conditions. Similarly, application of the fungicide led to a decrease of protease activity. For the highest dose of difenoconazole, there was a decrease in PHA activity of 56.98% for the soil samples maintained in field conditions. For the soil samples maintained in laboratory conditions, the PA activity increased by 40.24%. This study also found that the effects of the difenoconazole on the soil enzymes’ activities are dependent on temperature [56].

4.2. Myclobutanil

The effect of the myclobutanil on the DHA activity was evaluated using tea orchard soil. The fungicide was applied in concentrations of 0.1, 1, 2 and 10 mg/kg of soil and the sampling was carried out after 0, 5, and 10 days of incubation. The effect of the myclobutanil
applied to tea orchard soil on the activity of dehydrogenase was dependent on the dose of the fungicide and on the period of incubation. Application of the myclobutanil in a dose of 0.1 mg/kg of soil produced an increase in DHA activity, the highest activity being found in day 10. Application of the myclobutanil in doses of 1 and 10 mg/kg of soil produced a decrease in DHA activity for the first five days, and the DHA activity tended to recover at day 10 [34]. In another study, the myclobutanil was applied at a dose of 2 mg/kg of sandy loam tea orchard soil unamended and amended with fertilizers and the soil samples were incubated under environmental conditions for 12 months [57]. The DHA activity was assessed after one month and after 12 months for all the soil samples. The myclobutanil slightly inhibited the DHA activity after 30 days, but the effect disappeared in time because the pesticide had dissipated [57].

4.3. Paclobutrazol

The effect of the paclobutrazol on the DHA activity was assessed in a greenhouse experiment by treating samples of sandy loam soil with the fungicide in doses of 80 and 160 mg/kg of soil with an incubation period of 27 days. In the first week, the DHA activity decreased 23% and 44% for the doses of 80 and 160 mg/kg, respectively. There were no significant differences in the effects of the two doses of paclobutrazol after 27 days of incubation, the average decrease observed in the enzyme activity was 60% [33].

4.4. Propiconazole

One study performed by Satapute et al. (2019) [40] considered doses of 1.0, 5.0, 10.0, 15.0 and 20.0 kg/ha of propiconazole applied on both red sandy loam and deep black soil. The soil samples were incubated for 1–4 weeks in order to assess the effect of the fungicide on the activities of UA and PHA. The UA and PHA activities were enhanced in the first two weeks in both types of soil treated with propiconazole in doses of 1.0, 5.0 and 10.0 kg/ha but were slightly reduced after three weeks. The UA and PHA activities were significantly inhibited in the soil samples treated with 15.0 and 20.0 kg/ha doses of propiconazole. In all the treatments, the UA and PHA activities were higher in the deep black soil in comparison with the red soil during the incubation time [40].

4.5. Tebuconazole

Numerous studies found that the tebuconazole produced significant changes in the biochemical activity of the soil. The tebuconazole application in doses of 5 (the highest recommended field application dose), 50 and 500 mg/kg of chernozem calcic soil and incubated for 90 days led to suppression of the activity of the enzymes arylsulfatase, β-glucosidase, alkaline phosphatase, and urease [44]. After 90 days, the activities were 93.35%, 87.15% and 69.5% lower in the soil treated with the fungicide for urease, arylsulfatase and β-glucosidase activity, respectively, with no significant differences among the tebuconazole doses. Regarding DHA activity, it was inhibited by 14% when the tebuconazole was applied in a dose of 5 mg/kg [44]. Saha et al. (2016) performed a field study and evaluated the effect of the tebuconazole on the soil enzyme activities using black clay soil samples [58]. The tebuconazole was applied at the field rate (FR, 187.5 g/ha), 2-times FR (2FR) and 10-times FR (10FR). The results of this study indicated the inhibiting effect of tebuconazole applied in concentrations of 2FR and 10FR on the activities of DHA and nitrate reductase in soil from a field cultivated with peanuts (clayey in texture and highly calcareous). The activities of UA, PHA and aryl sulfatase were not affected or slightly inhibited, after which they recovered [58]. Quite similar results have been found in the study of Wang et al. (2016) [46]. The tebuconazole was applied in doses of 1, 10 and 100 mg/kg on fluvo-aquic soil, and the soil enzyme activities were measured after 0, 7, 30, 60, and 90 days of incubation. According to this study, the tebuconazole application in doses of 10 and 100 mg/kg had a negative effect on activities of DHA, UA, alkaline phosphatase, and invertase [46]. Bocmaga et al. (2019) [48] studied the effect of soil enzyme activity when the tebuconazole was applied in doses of 0.042, 0.083, 0.125, 1.249, and 2.499 mg/kg on loamy-sand soil for 60 days of
incubation. This study found that the tebuconazole in the concentration of 2.499 mg/kg of soil inhibited by 17.44% the activity of DHA, by 19.44% that of arylsulfatase, by 8.54% that of β-glucosidase and by 4.55% the activity of catalase. Another study investigated the effect of the tebuconazole on the soil enzymes in a greenhouse experiment on a sandy loam soil and using a foliar application of tebuconazole by spraying it on the leaves of spring barley. The tested concentrations were 0.046, 0.093 (the field rate), 0.139, 1.395, and 2.790 mg/plant, the incubation time was 60 days and the activities of soil enzymes were evaluated after 40 and 60 days, respectively [49]. The sprayed tebuconazole on leaves of spring barley affected the soil enzymes’ activities in a dose- and time-dependent manner. When the fungicide was applied in doses from 0.046 to 2.79 mg/plant, the activity of alkaline phosphatase was suppressed by 8.3% to 23.5%. When the fungicide was applied in doses of 1.395 and 2.79 mg/plant, the following data were found: (i) the DHA activity was decreased by 19.7% and 48.9%, respectively; (ii) the activity of catalase was decreased by 8.1% and 12.1%, respectively; (iii) the activity of urease decreased by 15.6% and 59.9%, respectively; and (iv) the activity of b-glucosidase decreased by 5.6% and 7.0%, respectively. The tebuconazole dose of 2.79 mg/plant produced a decrease of 37.3% of the activity of acid phosphatase. The tebuconazole has a stimulating effect on arylsulfatase; those activities increased by 16.6% to 26.6%. Furthermore, activities of these enzymes significantly changed with time. On day 40, the activities of all investigated enzymes were higher than on day 60, excepting that of the arylsulfatase. This decrease may be explained by the suppression of soil microorganisms by the fungicide contributing to lower enzyme secretion. The boosted activity of the arylsulfatase may be explained by the appearance of soil microorganisms that are characterized by high tolerance to tebuconazole [49].

4.6. Triadimefon

In a study performed by Singh (2005) [59], the triadimefon was applied in a concentration of 1 mg/kg of both mollisol and inseptisol soils. The samples were incubated for 30 days and the activity of PHA and DHA were evaluated. The PHA activity was not affected by the triadimefon application in either of the soils, but the DHA activity was significantly decreased by 70% and 50% in mollisol soil and inseptisol soil, respectively [59]. Another study, performed in laboratory conditions, assessed the effects of the triadimefon at different doses of 0.2, 0.5 (field rate) and 0.7 kg/ha on the enzymatic activities of soil microorganisms in red loamy soil from a tomato cultivated field [60]. There was an increase in the amylase activity for the application of the fungicide at field rate, but higher doses led to a decrease of the activity of amylase. A decrease in the activity of cellulase was found at all concentrations. In the case of invertase, there was a decrease in its activity after 24 h of incubation for all the triadimefon doses, but the activity recovered after 72 h. The DHA activity was stimulated by the presence of the triadimefon at all concentrations, the highest increase of DHA activity being found for the field rate [60].

The effects of the investigated fungicides on the activity of enzymes found in soil are time-dependent. A summary of the acute effects produced by high doses of triazole fungicides on the activities of enzymes found in soil is presented in Table 3.

Data presented in Table 3 reveal that usually high doses of fungicides contribute to the decrease of activities of numerous soil enzymes. This is strongly correlated with the effects of these fungicides on the microbial populations as the inhibition of activities of soil enzymes can occur due to a competition between microorganisms for limited carbon sources and/or antagonistic interactions between microorganisms [61].
Table 3. Effects of high doses of triazole fungicides on the activities of enzymes found in soil: red cells indicate decreased activities compared to the control, blue cells indicate increased activities compared to the control, green cells indicate no effects of the fungicides on the activity of enzymes, white cells correspond to the lack of available data: DHA—dehydrogenase activity, UA—urease activity, PHA—phosphatase activity, PA—protease activity, ASA—aryl sulfatase activity, NRA—nitrate reductase activity, β-GLCA—β-glucosidase activity, CA—catalase activity, IA—invertase activity, AA—amylase activity.

| Fungicide     | DHA | UA | PHA | PA | ASA | NRA | β-GLCA | CA | IA | AA |
|---------------|-----|----|-----|----|-----|-----|--------|----|----|----|
| Difenoconazole|     |    |     |    |     |     |        |    |    |    |
| Hexaconazole  |     |    |     |    |     |     |        |    |    |    |
| Myclobutanil  |     |    |     |    |     |     |        |    |    |    |
| Paclobutrazole|     |    |     |    |     |     |        |    |    |    |
| Propiconazole |     |    |     |    |     |     |        |    |    |    |
| Tebuconazole  |     |    |     |    |     |     |        |    |    |    |
| Triadimefon   |     |    |     |    |     |     |        |    |    |    |

5. Discussion

The scientific literature that we have identified reveals that the most studied triazole fungicides regarding their effects on the soil microbiota and on the activities of enzymes found in soil are the tebuconazole and the propiconazole, the most widely used fungicides in the United States in 2016 [62]. The tebuconazole was found to reduce the soil microbial biomass and activity, to affect the structure of the microbial communities, and to decrease the activity of numerous soil enzymes. The propiconazole was also found to decrease the total microbial population, to affect the structure of microbial communities, and to decrease the activities of urease and phosphatase. The information regarding the effects produced by metconazole, myclobutanil and triadimenol on the soil microbiota has not been reported. Regarding the effects of triazole fungicides on the activities of soil enzymes, the information has not been reported for cyproconazole, epoxiconazole, flutriafol, hexaconazole, metconazole, tetraconazole, triadimenol and triticonazole.

The information presented in this review reveals that the triazole fungicides, when used at the field rate, are not a risk to the environment. The overapplication of the triazole fungicides usually produces deleterious effects both on the population of microorganisms and on the activity of numerous soil enzymes. Information regarding the mode of action and target microorganisms of the triazole fungicides, added to information about their potential non-target effects on the soil microorganisms and on the activities of soil enzymes, should be considered when selecting the fungicide to be used in agricultural practices [54]. It assures the protection of the biological properties of the soil and optimizes the benefits resulting from the fungicide used. Furthermore, this type of synthesis should have multiple practical relevancies to a diverse variety of fields, including agriculture and food processing, environmental protection, forestry and biotechnology.

The literature data revealed that, used at the field rates, the triazole fungicides can be degraded by the soil microorganisms due to the recovery of microbial populations that are able to use them as food sources. The higher doses of fungicides usually led to reduction in the total microbial population and affected the structure of this population. The bacterial populations were affected by triazole fungicides but the mechanism is not yet understood.

The evaluation of the effects of the triazole fungicides on the population of soil microorganisms and on the activity of enzymes found in soil based on published information proved to be a difficult task, as the methodology, the results and their interpretation often differ from one study to another. Consequently, differing data have been reported regarding the effect of triazole fungicides on soil microbiota and on the soil enzyme activities. There are reported (for the same fungicide), distinct minimum doses that have deleterious effects on the soil characteristics, and it does not allow for clear conclusions. These divergent findings may be due to the fact that the studies were performed on various types of soil, some of them were done in laboratory conditions and the other in field conditions, or the periods of assessment and the methodologies employed were distinct. There are also other
factors that influence the experimental determinations: the temperature of incubation of the samples for laboratory conditions, the possible synergistic/antagonistic effects between the fungicide and adjuvants when the commercial formulations have been used, the method of soil management, etc. All these data reveal the necessity to establish a standard methodology comprising a broad spectrum analysis of soil microbial and enzymatic activities to be used when assessing the effects of pesticides on the soil health. Such a methodology should include the following data: the type of soil, the cropping history, the history of pesticides applications, the type of spraying equipment used, and the soil physicochemical properties (pH, temperature, electrical conductivity, the organic matter content, the contents of water, NH$_4$-N, NO$_3$-N and phosphate).

Currently, the control of many fungal diseases depends on the application of single site demethylation inhibitors such as triazole fungicides, and the omission of this group of fungicides due to environmental effects would have dramatic consequences for practical agriculture [61]. Consequently, measures are needed to ensure that their use does not adversely affect the environment. Measures that can be applied include: the use of a fungicide that has lower toxicity against non-target organisms, ensuring that the recommended doses are not exceeded, the application interval is observed, and that adequate soil management techniques are used (for example, removal of the infected plant material, cleaning of equipment used, crop rotation, plowing).

6. Conclusions

Even if some differing data have been reported in the scientific literature regarding the effects of the triazole fungicides on soil health, the information presented in this review illustrates that the known effects of the triazole fungicides on the soil health are dose and time dependent. These fungicides may impact the soil health either to their direct application against soilborne fungal pathogens and also indirectly by spraying them on foliar surfaces. High doses of the triazole fungicides greatly disturbs the structure of the microbial communities in soil and usually lead to the diminution of the soil microbial population and the decrease of the activities of enzymes found in soil. This illustrates the importance of following the recommended dose for each type of crop and/or soil so as to not produce long-term effects on the soil properties and activity. The biostimulating substances have been proven to be useful at reducing or neutralizing the adverse effect of these fungicides on soil microbial functions and biochemical processes. There is missing information concerning the effects produced by metconazole, myclobutanil and triadimenol on the soil microbiota and concerning the effects of cyproconazole, epoxiconazole, flutriafol, metconazole, tetraconazole, triadimenol, and triticonazole on the activity of soil enzymes.

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