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Abstract

The use of pesticides in agriculture has highly increased during the last 40 years to increase crop yields. However, today most pesticides are polluting water, soil, atmosphere and food. Pesticides are also impact soil enzymes, which are essential catalysts ruling the quality of soil life. In particular, the activity of soil enzymes control nutrient cycles, and, in turn, fertilization. Here, we review the effects of pesticides on the activity of soil enzymes in terrestrial ecosystems. Enzymes include dehydrogenase, fluorescein diacetate hydrolase, acid phosphatase, alkaline phosphatase, phosphatase, β-glucosidase, cellulase, urease and aryl-sulfatase. Those enzymes are involved in the cycles of carbon, nitrogen, sulfur and phosphorus. The main points of our analysis are (1) the common inhibition of dehydrogenase in 61 % of studies, stimulation of cellulase in 56 % of studies and no response of aryl-sulfatase in 67 % of studies. (2) Fungicides have mainly negative effects on enzymatic activities. (3) Insecticides can be classified into two groups, the first group represented by endosulfan having an overall positive impact while the second group having a negative effect. (4) Herbicides can be classified into two groups, one group with few positive effect and another group with negative effect.

Keywords

Pesticides, Soil, Enzyme activity, Microbial diversity, Microcosm studies

Introduction

Pesticides are widely used in crop production and are known to induce major environmental problems in Europe. With an increased pesticide use, questions are rising on potential effects regarding public health and environment. Pesticides pollute air, soil, water resources and contaminate the food chain. The European Commission has taken into consideration this problem by publishing the Directive 2009/128/EC, which establishes a framework to achieve a sustainable use of pesticides. This directive aims at reducing the risks and impacts of pesticide use on human health and environment by promoting the use of integrated pest management and alternative approaches or techniques such as non-chemical alternatives to pesticides.

Although the Directive 91/414/EEC of 15 July 1991 on the authorization of plant protection products has already resulted in the elimination of more than 75 % of active substances from the market according to their ineffective- ness or toxicity (Karabelas et al. 2009), a large variety of active molecules are still used. Over 150 different pesticides are currently authorized in member states of the European Union. In agriculture, these compounds are applied to improve crop yield and quality. Globally, about 140.000 tons of pesticides are applied annually in the Europe (Eurostat 2007). The European farmers devote a significant portion of their budget to buy these agrochemicals. Usually, several pesticides are required during a cropping season, thus agricultural soils often contain a mix of different pesticides at different concentrations.

Pesticides include diverse groups of inorganic and organic chemicals. They are divided into groups according to their primary target and include herbicides, insecticides, nematicides, fungicides and soil fumigants (Gevao et al. 2000). Pesticides can be classified in a number of ways: by target pest, their mode of action or chemical family. The main chemical groups are organochlorine, organophosphate, carbamate, pyrethroids, triazine and sulfonylurea (Afify et al. 2010). There are a large variety of pesticides available on the market, and chemical families are more diversified than proposed by these authors (Table 1). Some chemical families of pesticides can be efficient as fungicide, herbicide and insecticide, which make it difficult to classify them.

The interaction between soil components and pesticides influences the biochemical processes driven by microorganisms. Telluric fungi (Hernandez-Rodriguez et al. 2006; Ronhede et al. 2007) and bacteria (Dong et al. 2005; Qiu et al. 2006) are able to degrade or mineralize pesticides via enzymatic reactions. Pesticides' effects on soil microorganisms can be

determined by the study of functional parameters such as carbon and nitrogen mineralization that are governed by enzymatic activities. Those activities play an important role because all biochemical transformations in soil depend on or are related to the presence of enzymes. They are indicators of biological equilibrium (Frankenberger and Tabatabai 1991), fertility (Schuster and Schroder 1990a b; Antonious 2003) and changes in the biological status due to soil pollution (Nannipieri and Bollag 1991; Kucharski and Wyszkowska 2000; Trasar- Cepeda et al. 2000; Chu et al. 2003; Bending et al. 2004). Finally, the measurement of specific enzymatic activities may contribute to understand the metabolic processes involved in the biogeochemical cycles of nutrients. Pesticides reaching the soil may disturb local metabolism or enzymatic activities (Engelen et al. 1998; Liu et al. 2008; Hussain et al. 2009). Negative impacts of pesticides on soil enzymes such as hydrolases, oxidoreductases and dehydrogenase activities have been widely reported in the literature (Perucci and Scarponi 1994; Ismail et al. 1998; Malkomes and Dietze 1998; Monkiedje and Spiteller 2002; Monkiedje et al. 2002; Menon et al. 2005; Caceres et al. 2009). There is also evidence that soil enzymes may pro- vide valuable general information on transformation of pesticides in soils (Gianfreda and Bollag 1994; Kalam et al. 2004; Gil-Sotres et al. 2005; Hussain et al. 2009).

Once a pesticide is released into the environment, understanding its behavior becomes of major scientific interest. Its evolution is strongly linked to soil's microbiological composition and especially to soil enzymatic activities. The diversity of pedoclimatic contexts, pesticides' nature and experimental protocols (applied doses) in scientific literature make data analysis extremely complex (Gevao et al. 2000). Nevertheless, the literature in this area is abundant and the authors often provide findings that suggest that enzymatic activities could be indicators of soil use and management because of their relationship to soil biology (Yao et al. 2006). Soil enzymatic activities are assumed to be early indicators of soil degradation compared to chemical or physical parameters (Dick et al. 1994). Among soil enzymatic activities, hydrolases are the most commonly measured activities in soils and therefore proposed by many authors as potential indicators of soil state (Tabatabai 1994; Deng and Tabatabai 1996; Dick et al. 1996; Floch et al. 2011).

The main aim of this work is to summarize and analyze the rich data accumulated in the literature during the last years, concerning pesticide effects on soil enzymatic activities: dehydrogenase and a large range of hydrolases. This review attempts to find out or identify common determinants explaining variation patterns of soil enzymatic activities in relationship with application of different types of pesticides. The patterns of soil enzymatic responses could

be then used to reflect experimental designs (most relevant enzymatic activities to be monitored, pesticide doses to be applied…) aiming at under- standing the impact of pesticides on soil microbes. More generally, they could provide valuable support for decision-making (farmers, advisers, public authorities) concerning the choice of pesticides, which are less harmful for soil microorganisms.

Here, we review the effects of pesticides on enzymatic activities and we discuss trends of response for each soil enzymes. We also analyze the possible relationships between the responses of soil enzymatic and mechanisms action pesticides.

Effect of pesticides on soil enzymatic activities

The literature concerning the effect of pesticides on soil enzymatic activities is abundant and sometimes discordant. Some studies were realized in field while others were performed in natural microcosms. In the latter case, soil preparation and incubation times were very variable in function of authors. Thereby, in order to achieve a comparative analysis, we have chosen to examine only the data that refer to microcosm experiments (Fig. 1). These con- trolled experiments have indeed the advantage to provide more robust results; the small ecosystem size enables high replication in experiments (Srivastava et al. 2004). Soil microcosms allow also observing the effects of only one pesticide on soil enzymatic activities and not the synergic or antagonist effects of molecules, as it is often the case in field studies where it is difficult to dispense of cumulative effects of pesticides resulting of agricultural practices. Moreover, only the results observed between 28 and 50 days of incubation were reported here, in order to avoid considering transient effects of pesticides on enzymatic activities.

The synthesis of the review is reported in Table 2. It highlights that the enzymes studied are either indicators of overall microbiological activity, or specific hydrolases of carbon, nitrogen, phosphorus and sulfur biochemical cycles. In the different studies, the applied doses of pesti- cides were given in reference to the field rate (FR, i.e., the approved dose). It appears that many studies used 10, 100 up to 1,000 times the recommended field dose in order to simulate repeated application or long-term use of pesticides, even if this method is questionable.

Global metabolic activities

Dehydrogenase activity

Dehydrogenase occurs in all living microbial cells, and it is linked with microbial respiratory processes (Bolton et al. 1985). This intracellular enzyme is an indicator of overall microbial activity of soils. The impact of pesticides on dehydrogenase activity has been widely reported in the literature. Pesticides generally appear to have an adverse effect on dehydrogenase activity (Table 2). The majority of insecticides are either neutral toward this activity (Caceres et al. 2009) or they inhibit it (Beulke and Malkomes 2001; Kalam et al. 2004; Yao et al. 2006; Jastrzebska 2011). Only endosulfan seems to stimulate dehydrogenase activity when it goes 100 to 200 times the standard rate of application (Kalyani et al. 2010; Defo et al. 2011). But con- tradictory results show no effect of endosulfan at 200 times the regular dose in soil with pH 4.8. Likewise, herbicides, except butachlor (Min et al. 2002; Xia et al. 2011), have a repressive effect on dehydrogenase activity, whatever conditions of application, including dose and soil pH (Beulke and Malkomes 2001; Bennicelli et al. 2009; Sebiomo et al. 2012). In contrast, it was not possible to identify a single type of response of this activity to fungicides: the enzyme was alternately stimulated and inhibited. Authors suggested that the absence of effect on dehydrogenase activity was probably due to the time of incubation, which was insufficient to induce any effect. Nevertheless, the effect of a pesticide on soil microorganisms is governed not only by the chemical and physical properties of the pesticide itself, but also by the soil type, soil properties, and prevailing environmental conditions (Dick et al. 2000). The dehydrogenase was severely inhibited at higher doses of fungicides (Monkiedje et al. 2002; Bello et al. 2008).

Generally, whatever the dose considered, fungicides, herbicides and insecticides show inhibitory effects or no effects on the dehydrogenase activity, except endosulfan and mancozeb.

Fluorescein diacetate hydrolase

The fluorescein di-acetate hydrolase activity has the potential to broadly represent soil enzymatic activities and accumulated biological effects. Fluorescein di-acetate is a substrate hydrolysed by a number of different enzymes, such as protease, lipase and esterase and its

hydrolysis was observed among a wide array of primary decomposers, bacteria and fungi (Janvier et al. 2007). In comparison with dehydrogenase, only few studies have examined the response of fluorescein diacetate hydrolase activity to the presence of pesticides in soil. There is no clear answer of fluorescein diacetate hydrolase to pesticides' input, but it seems to be more influenced by insecticides (Das et al. 2007; Bishnu et al. 2012) than herbicides (Perucci et al. 2000; Zabaloy et al. 2008). Fluorescein diacetate hydrolase activity was stimulated by the supply of pesticides of imidazolines (Imazethapyr) and organochlorines (endosulfan) families (Perucci et al. 2000; Kalyani et al. 2010), which have been less studied than other families of pesticides. The application of organophosphate (chlorpyrifos and ethion) at different doses had the same effect on this enzymatic activity (Dutta et al. 2010; Bishnu et al. 2012).
Unfortunately, the number of experimentations carried out was not sufficient to draw any conclusion. It would be interesting to examine whether the response of fluorescein diacetate hydrolase is the same with more represented organophosphate pesticides. Slight and transitory increases in fluorescein diacetate hydrolase activity were observed at the highest applied pesticide rates (tenfold field rate).
Moreover, the author proposed a new synthetic index, the specific hydrolytic activity, to assess microbial activity in reply to xenobiotic treatments, and he considered fluores- cein diacetate hydrolase as a suitable tool for measuring the early detrimental effect of pesticides on soil microbial biomass, as it is a sensitive and non specific test, which is able to depict the hydrolytic activity of soil microbes. No study on the effects of fungicides on fluorescein diacetate hydrolase activity has been identified in this review. Fluorescein diacetate hydrolase activity in soil is poorly influenced by herbicides or insecticides applications, except endosulfan applications, which seems to stimulate this activity.

Carbon cycle enzymatic activities

Cellulase and β-glucosidase

β-Glucosidase and cellulase are very important enzymes involved in the transformation/decomposition of organic matter in soil. Their final product is glucose, an important carbon energy source for soil microorganisms (Deng and Tabatabai 1994).
Very few references are available since the year 2000 concerning the effect of fungicides on

cellulase activity. Indeed, only two fungicides have been tested and seem to have no pronounced negative effects on this activity (Niemi et al. 2009). Likewise, the herbicides from different chemical families (urea, triazine and nitrile) seem to have no effect on cellulase activity even with 10 times the dose of application (Omar and Abdel-Sater 2001; Niemi et al. 2009). The insecticides are more represented, particularly molecules, which belong to the family of organophosphate (monocrotophos, quinalphos, profenofos and selectron). They have globally a stimulating effect on this enzymatic activity (Omar and Abdel-Sater 2001; Gundi et al. 2007; Niemi et al. 2009). These molecules may destroy soil insects and make substrates available to stimulate cellulase activity. Moreover, Gundi et al. (2007) showed that there was a link between enzymatic activity of cellulase in soils and cellulolytic fungi in the presence of insecticides. Indeed Populations of cellulolytic bacteria in both the black vertisol and red alfinsol soils were enhanced with increasing concentration of monocrotophos, quinalphos and profenofos. The cellulase activity is either inhibited or insensitive to the fungicides and herbicides tested, while the various insecticides tested, except Selectron, have a stimulatory effect on the enzyme's activity.

According to the data presented in Table 2, the activity of β-glucosidase shows two patterns of variation in the presence of pesticides: it is either inhibited or stays unchanged. The fungicides from amides' family (mefenoxam et metalaxyl) affect the activity of the β- glucosidase (Monkiedje et al. 2002). However, the combined supply of a fumigant (methyl bromide) and a fungicide (chloropricin) has no effect on this activity. It was observed that herbicides have no effect on β-glucosidase activity, even when using molecules, which belong to different herbicides' families (linuron and metribuzin) (Niemi et al. 2009). On the contrary, it appears that glyphosate and diflufenican applied to a same soil inhibit the β-glucosidase activity (Tejada 2009). β-glucosidase activity do not show a clear response to insecticides, except endosulfan, which seems to stimulate the activity of β-glucosidase at 200 times the usual dose (Defo et al. 2011). It seems that the β-glucosidase activity is either inhibited or insensitive to the application of pesticides, whether fungicides, insecticides or herbicides, except for the results observed by Defo et al. (2011) with endosulfan on acid soil. This observation may be related to the strong functional redundancy of β-glucosidase activity, since a large number of microbial species, whether fungi or bacteria, is able to express this enzymatic activity.

Phosphorus cycle enzymatic activities

Acid phosphatase, alkaline phosphatase and phosphatases

Phosphatases include five major groups of enzymes: The phosphomonoesterases, the phosphodiesterases, the phosphotriesterases, the pyrophosphatases and the phosphoamidases. Among these enzymes, phosphomonoesterases are the most abundant in soils, probably due to the low substrate specificity of this group of enzymes (De Cesare et al. 2000). Phosphomonoesterases include acid and alkaline phosphatase, which can be distinguished according to the optimum pH for their activity. In literature, some authors use the term phosphatase without distinguishing between acid and alkaline phosphatase. Phosphatases are a broad group of enzymes that are capable of catalysing hydrolysis of esters and anhydrides of phosphoric acid (Schmidt and Weary 1962; Schmidt et al. 1962). In soil ecosystems, these enzymes are believed to play a critical role in Phosphorus cycle (Schneider et al. 2001). Experi- ments show that they are correlated to phosphorus stress and plant growth. Apart from being good indicators of soil fertility, phosphatase enzymes play a key role in the soil system (Eivazi and Tabatabai 1977; Dick and Tabatabai 1987; Dick et al. 2000). The effect of pesticides on phosphatases, in particular on acid and alkaline phosphatase activities (when the distinction is made by the authors), is given in Table 2. Several researchers have shown either unchanged or decreasing phosphatase activity following various pesticide applications (Kalam et al. 2004; Yan et al. 2011). Acid and alkaline phosphatases are mostly found in microorganisms and animals (Tabatabai 1980). It appears that the supply of fungicides inhibits alkaline phosphatase activity (Rasool and Reshi 2010; Sharma et al. 2010), whereas it stimulates the activity of acid phosphatase. This observation is more pronounced in the study conducted by Monkiedje et al. (2002), which showed that the application of mefenoxam and metalaxyl fungicides in soil at pH 7.2 inhibited alkaline phosphatase activity and stimulated acid phosphatase activity. Regarding the phosphatases, only the fungicide metalaxyl seems to stimulate this group of enzymes (Sukul 2006). Other fungicides either had no effect or inhibited their activity (Bello et al. 2008; Tejada et al. 2011; Yan et al. 2011). Regarding the herbicides, most of the time the response of acid and alkaline phosphatase activity is similar, and the two activities are either stimulated (imazethapyr) or remain unchanged (aurora 40WG and rimsulfuron) (Perucci et al. 2000; Omar and Abdel-Sater 2001; Bacmaga et al. 2012). Butachlor appears to stimulate the activity of alkaline phosphatase (Xia et al. 2011). Herbicides

severely inhibit phosphatase activities even when they are applied in very different conditions in regard to the pesticides dose and soil physicochemical properties (Min et al. 2001; Tejada 2009). The enzymatic activities of acid and alkaline phosphatase respond differently to insecticides. Indeed, the same insecticide may inhibit acid phosphatase and stimulate alkaline phosphatase activity, and vice versa (Omar and Abdel-Sater 2001; Cycon´ et al. 2010; Defo et al. 2011; Jastrzebska 2011). The difference in behavior of both acid and alkaline phospha- tases toward pesticides can be attributed to the structure of soil microbial communities and their sensitivity to pesti- cides applications (Klose et al. 2006). Insecticides had inhibitory effects on phosphatases (Madhuri and Rangaswamy 2002; Yao et al. 2006). However, the cadusaphos (organophosphate insecticide) applied at 10 times the recommended dose seemed to have no effect on phosphatase activities (Vavoulidou et al. 2009). Overall, pesticides appear to have an inhibitory effect on the enzymatic activities involved in the phosphorus cycle.

Nitrogen cycle enzymatic activity

Urease

Urease is an enzyme that catalyses the hydrolysis of urea into carbon dioxide and ammonia and is a key component in the nitrogen cycle in soils. Due to this role, urease activities in soils have received a lot of attention since it was first reported by Rotini (1935), a process considered vital in the regulation of nitrogen supply to plants after urea fertilization (Makoi and Ndakidemi 2008). Soil urease originates mainly from plants (Polacco 1977a, b) and microorganisms. It is found both as intra- and extra-cellular enzyme (Blakeley and Zerner 1984; Burns 1986; Mobley and Hausinger 1989). Most of the referenced studies reported that herbicides and fungicides appear to have no effect (Cycon´ et al. 2010; Romero et al. 2010; Tejada et al. 2011; Yan et al. 2011; Bacmaga et al. 2012) or reduced effect on urease activity (Sukul 2006; Caceres et al. 2009; Tejada 2009). Decreased urease activity in soil due to the application of pesticides reduces urea hydrolysis, which is generally beneficial, because it helps to maintain nitrogen availability to plants (Antonious 2003). On the contrary, the fungicides carbendazim and validamycin enhanced urease activity, respectively, up to 70 % and to 13-21 % (Qian et al. 2007; Yan et al. 2011). The urease activity appears to be either unaffected or inhibited by the addition of pesticides except carbendazim and validamycin, which tend to stimulate this enzyme activity. Thus, it is difficult to identify a clear response of this enzymatic

activity to pesticides because this enzyme has received little attention during the last 10 years.

Sulfur cycle enzymatic activity

Arylsulfatase

Arylsulfatases are typically widespread in soils (Tabataba and Bremner 1970b, c; Gupta and Germida 1988; Ganeshamurthy and Nielsen 1990; Ganeshamurthy and Takkar 1997). They are responsible for the hydrolysis of sulfate esters in the soil (Kertesz and Mirleau 2004) and are secreted by bacteria into the external environment as a response to sulfur limitation (McGill and Cole 1981).The effects of pesticides on arylsulfatase activity in soil are poorly documented from the year 2000. Generally, the pesticides do not seem to affect the activity of this enzyme (Niemi et al. 2009; Tejada 2009; Vavoulidou et al. 2009). The endosulfan (insecticide) applied at elevated level (100 ppm) increased significantly arylsulfatase activity. This increase in arylsulfatase activity was transitory and declined with the depletion of applied endosulfan. The short-term effect of endosulfan may be due to its degradation or its gradual adsorption by the soil colloid, making it unavailable for microbes (Kalyani et al. 2010). The same ephemeral effect on arylsulfatase was observed with the fungicide metalaxyl (Sukul 2006). It is difficult to identify a clear response of this enzymatic activity to pesticides for the same reasons as for urease.

Pattern of variation of enzymatic responses to pesticides

Interactions between pesticides and soil enzymes depend on several factors. Concerning pesticides, persistence, mode of inhibition, toxicity level, concentration and bioavailability are all factors able to influence soil enzyme activities (Schaffer 1993; Gevao et al. 2000). In the same way, physicochemical and biological soil characteristics, as well as mode of synthesis, expression and inhibition of enzymes are able to modify the expression of soil functions (Zimmerman and Ahn 2011). One way to take into account all these parameters is to realize a global analysis of the literature, with the aim to identify response profiles of enzyme activities subject to pesticide exposure.

In order to identify main patterns of enzymatic responses to addition of pesticides, the previous data were re-analyzed. Thus, based on data of all articles cited in this review, percentages of

positive, neutral or negative responses were calculated for each enzymatic activities regardless of pesticide (Table 3). Patterns of responses seem to emerge. Indeed, among hydrolases acid phosphatase, alkaline phosphatase, phosphatases and urease are overall inhibited by pesticides. This inhibition effect is also observed for dehydrogenase activity and is more pronounced whatever pesticides given the number of studies measuring this enzyme. Fluorescein di-acetate hydrolase and arylsulphatase activities are unaffected by pesticide addition. On the contrary, in most cases, cellulase is activated by pesticides and especially by insecticides. The β-glucosidase is the only enzyme of this analysis that has not an evident response. Both negative and no significant effect is recorded for the activity. The absence of pattern of variation for b-glucosidase is probably due to the high redundancy of this activity in microbial communities. These patterns should be taken with caution due to simplifications needed to reach a generalization; in our case, the variation of pH, field dose and pesticide type were not take account.

A previous review led the author to conclude that the patterns of enzymatic responses were difficult to define in function of pesticide applications. This author had used published results without attempting to standardize their expression in particular the choice of a time step for the observation of the effect or the selection of studies with the closest incubations conditions (here sieved soil). It is therefore consistent to find contradictory results.

Relationships between pesticide mechanisms of action and enzymatic responses

The understanding and interpretation of enzymatic responses after pesticides' addition are very difficult. Indeed, the observed responses are the resultant of numerous factors. There are direct and/or indirect interactions of pesticides with soil enzymes (Gianfreda and Rao 2008). Among them, it can be cited the binding of pesticide with the active site of the enzyme which affect their catalytic activities (Tabatabai 1994) or the use of pesticides as a nutriment source by the microorganisms which may shift not only the balance between the communities but more directly the biosynthesis of enzymes by induction or repression phenomen (Cycon et al. 2006; Tejada 2009; Zabaloy et al. 2012; Chishti et al. 2013). To these, direct phenomena must also be added the indirect impacts of pesticides on microbial community structure which lead to changes in soil enzymatic activities (Bjornlund et al. 2000; Singh and Walker 2006; Lo 2010). These impacts are strongly related to functional redundancy of the target activity (Chaer et al. 2009; Griffiths and Philippot 2013; Puglisi et al. 2012) and the intrinsic properties of soil, pH,

humus, clay content or organic matter that influence the accessibility of pesticides (Chen et al. 2001; Gundi et al. 2007; Defo et al. 2011; Munoz-Leoz et al. 2013). At present, we lack the necessary information on how these different phenomena interact in order to predict a general response for a given enzyme. Moreover, the diversity of experimental conditions (various soils, nature and application rates of pesticides, time after exposure, target enzyme) make comparisons difficult, even if we took the precaution to consider only incubation studies in microcosms under controlled conditions. Finally, the impact of differences between methodological approaches should not be forgotten as reported by Burns et al. (2013). Sample preparation may differ in soil/substrate ratio, pH of the buffer, time and temperature of incubation, soil preparation (sieved or not) and soil storage (fresh, dried or frozen), soil pre-treatment (chemicals), assessment of both intra- and extracellular enzymes, or only extracellular enzymes (Dick et al. 2000). Moreover, the assay methods used to measure in situ enzymatic activities could be very different based on Para-nitro phenyl substrates adapted from (Tabataba and Bremner (1970a) or on microplate fluorimetry assays using the fluorescent compound 4-methylumbelliferone (Marx et al. 2001; Pritsch et al. 2004; Drouillon and Merckx 2005; Niemi and Vepsalainen 2005; Winding and Hendriksen 2007; de Forest et al. 2009; Trap et al. 2012).

Through the analysis of the published works reported in this review, we have tried to underline potential relation- ships between the action mechanisms of pesticides and responses of soil enzymatic activities. To that purpose, the same method as above was applied, i.e., percentages of positive, neutral or negative responses were calculated for each mechanism of action whatever enzymes. Mechanisms of action where the number of experiments was \9 were excluded (Fig. 2). The general trends highlighted and reported in Fig. 2 have been confronted with numerous results from other articles dealing with the impact of pesticides on microbial communities.

Concerning the fungicides, both mechanisms of action presented in Fig. 2 seem to induce an overall negative response of enzymatic activities. Indeed, fungi are responsible for mineralization of organic matter in soil and release of available carbon; hence, their disturbance by fungicides may have a harmful impact on microbial communities and their activities. Incidentally, several authors showed under microcosm experiments that fungicides applications at higher rates than recommended had deleterious effects on fungal populations, while the bacterial populations increased (Monkiedje and Spiteller 2002; Moharram et al. 2004; Strickland et al. 2004; Cycon et al. 2006; Bending et al. 2007; Cycon et al. 2010). This

switch between fungal and bacterial communities is also explained by the increased levels of nutrients and energy sources released from dead fungal hyphae (Cycon et al. 2006; Tejada et al. 2011). Moreover, Munoz-Leoz et al. (2011) demonstrated a decrease in microbial biomass in parallel to the decrease in enzymatic activities after fungicide application. This imbalance of microbial populations may lead to the global negative response of enzymatic activities regarding an incubation time range from 28 to 50 days. In field conditions, many authors found the same negative trend on soil enzymes (Niewiadomska 2004; Niewiadomska and Klama 2005) even at recommend field rate after only 3 years of fungicide treatments (Niemi et al. 2009).

For the insecticides, our bibliographic analysis showed that the global enzymatic response is different according to the mechanism of action. Insecticides that altered the movement of ions across the nerve cell membranes induce rather a positive response of soil enzymatic activities while insecticides inhibiting the enzyme acetylcholinesterase of nerve impulses caused rather a negative response (Fig. 2). Organochlorine and particularly endosulfan, responsible for global positive responses, are the most commonly and widely used insecticides worldwide. With the application of endosulfan, an increase in microbial biomass carbon is observed (Kalyani et al. 2010). This result may be the consequence of bacterial biomass increase despite fungal biomass may be reduced (Joseph et al. 2010; Xie et al. 2011). Moreover, whatever the impact of endosulfan on the equilibrium of microbial population, many authors reported the ability of telluric bacteria or fungi to degrade this insecticide which could induce an increase in microbial biomass and/or an activation of enzymatic production (Bhalerao and Puranik 2007; Kataoka et al. 2010, 2011; Castillo et al. 2011; Yu et al. 2012). On the contrary, insecticides of the organophosphate family create an overall inverse response of enzymatic activities compared to endosulfan. Indeed, chlorpyrifos, which is representative of this insecticide type widely studied, decreased the soil microbial biomass, bacterial, fungal and actinomycetes populations at a concentration corresponding to 20 time the field recommended rate (Shan et al. 2006; Vischetti et al. 2007). A same result was found for another molecule, monocrotophos (Zayed et al. 2008), and a field experiment confirmed this alteration of microbial community structure and functions (Susan et al. 2004). However, other molecules of organophosphate group had no adverse effects on soil bacterial and fungal counts (Martinez-Toledo et al. 1992; Tejada 2009) or increased slightly these microbial populations (Das and Mukherjee 2000). The results of the literature concerning the study of insecticides on microbial communities are consistent with the major trends built from the analysis of 50 articles.

Finally, concerning herbicide effects on soil enzymes based on their mechanism of action show both negative and neutral responses (Fig. 2). Herbicides that inhibit the acetolactate synthase enzyme and photosynthesis process have predominately neutral effect on soil enzymatic activities. The results of the literature are consistent with the observed trends. The addition of atrazine (Radivojevic et al. 2008) or metsulfuron-methyl herbicides (Zabaloy et al. 2008) induced, respectively, no effect or minor changes to soil microbial activity, bacterial density and functional richness. Effect of long-term atrazine and metolachlor applications at fields demonstrated also that structure of total bacterial community and Acidobacterium, Actinomycetes, methanotroph groups were not severely affected (Seghers et al. 2003). Herbicides that inhibit the 5-enolpyruvylshikimate-3-phosphate synthase are widely represented by glyphosate. The application of this molecule leads to negative responses of soil enzymatic activity in 77 % of experiments reported in this review (Fig. 2). Studies on abundances and structures of microbial communities of soil or rhizosphere demonstrated that recommended field rate of glyphosate had a benign effect (Barriuso and Mellado 2012; Hart et al. 2009) while at a high concentration were observed a short-term stimulation of bacteria (Ratcliff et al. 2006; Weaver et al. 2007). These microbial structure data are in opposition with the results of the studies used to build Fig. 2. In fact, the authors who observed the decrease in enzymatic activities described in parallel an impact on microbial communities. This observation weakens the hypothesis of a potential spread of observations whatever the context.

Conclusion

During last decades, pesticides were increasingly used in agriculture in order to limit crop diseases and increase food production. Today we find increasing amounts of pesticides in the different environmental compartments: water, air and soil. Several investigations have been devoted to study the effect of pesticides on a few parameters of soils. More recently, there have been a number of publications, which recommended soil microcosm, or terrestrial model ecosystem approaches, to assess the effect of pesticides on multiple ecological process. It is difficult to understand the role of pesticides in perturbing the microbial communities and their enzymatic activities in soil due to divergent research findings reported in the literature. A number of factors could be responsible for those controversial results such as soil properties, chemical nature and concentration of pesticides, biological function observed. Even if pesticides applied at recommended rates may cause slight and transient changes to populations

or activities of soil microorganisms (Johnsen et al. 2001), it is obvious that long-term recurrent applications of pesticides are known to interfere with the biochemical balance, which can reduce soil fertility and productivity by affecting local metabolism and enzymatic activities. To preserve the environment, many of those molecules have been and will be withdrawn from the market such as clothianidine, imidaclopride, thiame´thoxame and endosulfan. This work has allowed to (i) identify patterns of enzymatic activity response to pesticides' application, (ii) link them with the pesticides' mechanisms of action, (iii) classify the pesticides according to their stimulating, inhibiting or neutral effects on enzymatic activities (iv) bring overall trends face to face with literature related to pesticides impacts on microbial com- munities. Those observations must be considered with care in regard with the number of papers analyzed and the approach used to make conclusions. However, the diversity of contexts and approaches that constitute the basis of this analysis can strengthen our conclusions.

The case of glyphosate weakens the hypothesis of a potential spread of observations whatever the context. Nevertheless, the other general trends described appeared to be confirmed. These first patterns of response have to be validated by further studies which may rely on the development of new technologies such as sensitive molecular- based approaches for measuring microbial community structure (e.g., pyrosequencing), as well as the use of real-time PCR or proteomic approaches to evaluate the expression level of genes involved in key ecological functions. For researchers, the design of experiments related to pesticide effects on soil functioning is difficult especially regarding the choice of the enzymatic activities to be monitored, the pesticides family and rates to be applied, or the intrinsic parameters of soils. Finally, in this review, we attempt to look for general trends of enzymatic responses to pesticides, which could be useful for researchers and thus for policy decision markers in order to replace agronomy in the center of agriculture.

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Table 1. Examples of pesticides used in agricultural systems

Type	Chemical family	Mode of action	Pesticides
Fungicide	Amide	Phenylamides affect RNA synthesis	Mefenoxam
	Amide	Phenylamides affect RNA synthesis	Metalaxyl
	Antibiotic	Inhibition of peptidyl transferase enzymes	Chloramphenicol
	Antibiotic	Inhibits enzyme trehalase	Validamycin
	Benzimidazole	Inhibition of tubulin formation	Benomyl
	Benzimidazole	Inhibition of tubulin formation	Carbendazim
	Dinitroanilines	Disturb the production of energy in the fungus	Fluazinam
	Carbamates	Affect respiration process and production of energy	Mancozeb
	Imidazol	Disturb membrane function	Prochloraz
	Organochlorine	Affect respiration process and production of energy	Chlorothalonil
	Organochlorine	Affect respiration process and production of energy	Pentachlorophenol
	Organochlorine	Affect respiration process and production of energy	Trichlorophenol
	Phthalimide	Inhibiting respiration of numerous species of fungi	Captan
	Strobilurins	Quinone outside Inhibitors	Azoxystrobin
	Triazoles	Inhibition of demethylation in sterol biosynthesis	Propiconazole
	Triazoles	Inhibition of demethylation in sterol biosynthesis	Tebuconazole
Herbicide	Amide	Inhibition of carotenoid biosynthesis	Diflufenican
	Chloroacetanilide	Biosynthesis inhibitor which has a multiple-site	Butachlor
	Chloroacetanilide	Inhibition of mitosis and cell division	Metazachlor
	Dinitroanilines	Inhibition of mitosis and cell division.	Trifluralin
	Dinitrophenol	Disturb photosynthesis process	Dinoterb
	Glycine	Inhibitors of 5-enolpyruvylshikimate-3-phosphate synthase	Glyphosate
	Imidazolinone	Inhibition of acetolactate synthase (ALS)	Imazethapyr
	Nitriles	Inhibitors of photosynthesis at photosystem II Site A	Bromoxynil
	Sulfonylurea	Inhibition of acetolactate synthase	Rimsulfuron
	Sulfonylurea	Inhibition of acetolactate synthase	Aurora 40 WG
	Sulfonylurea	Inhibition of acetolactate synthase	Metsulfuron-methyl
	Triazine	Inhibitors of photosynthesis at photosystem II Site A	Atrazine
	Triazine	Inhibitors of photosynthesis at photosystem II Site A	Metribuzin
	Triazine	Inhibitors of photosynthesis at photosystem II Site A	simazine
	Triazinone	Inhibitors of photosynthesis at photosystem II Site A	Primextra
	Ureas	Inhibitors of photosynthesis at photosystem II Site B	Diuron
	Ureas	Inhibitors of photosynthesis at photosystem II Site B	Linuron
Insecticide	Benzoylphenylurea	Chitin Synthesis Inhibitor	Novaluron
	Carbamates	Affect respiration process and production of energy	Methomyl
	Chloronicotinyl	Acetylcholine receptor agonists/antagonists	Acetamiprid
	Organochlorine	Alter the movement of ions across the nerve cell membranes	Dieldrin
	Organochlorine	Alter the movement of ions across the nerve cell membranes	Endosulfan
	Organophosphate	Inhibition of the enzyme acetylcholinesterase of nerve impulses	Cadusaphos
	Organophosphate	Inhibition of the enzyme acetylcholinesterase of nerve impulses	Chlorpyrifos
	Organophosphate	Inhibition of the enzyme acetylcholinesterase of nerve impulses	Dichlorvos
	Organophosphate	Inhibition of the enzyme acetylcholinesterase of nerve impulses	Ethion
	Organophosphate	Inhibition of the enzyme acetylcholinesterase of nerve impulses	Fenamiphos
	Organophosphate	Inhibition of the enzyme acetylcholinesterase of nerve impulses	Monocrotophos
	Organophosphate	Inhibition of the enzyme acetylcholinesterase of nerve impulses	Phorate
	Organophosphate	Inhibition of the enzyme acetylcholinesterase of nerve impulses	Profenofos
	Organophosphate	Inhibition of the enzyme acetylcholinesterase of nerve impulses	Quinalphos
	Organophosphate	Inhibition of the enzyme acetylcholinesterase of nerve impulses	Selectron
	Pyrethroids	Interfere with the sodium channels of nervous system	Cypermethrin

Table 2. Effect of pesticides on soil enzyme activities in different experimental conditions

Enzymes	Pesticides	Type	Pesticides doses	pH of soil	Effect of pesticide between 28 and 50 days of incubation	References
Global enzymatic activities						
Dehydrogenase	Azoxystrobin	F	FR	6.5	−	Bending et al. (2007)
	Benomyl	F	FR	4.7	+	Chen et al. (2001)
	Benomyl	F	FR	7.2	+	Chen et al. (2001)
	Captan	F	FR	4.7	−−−	Chen et al. (2001)
	Captan	F	FR	7.2	+	Chen et al. (2001)
	Carbendazim	F	9*FR	4.4	−	Burrows and Edwards (2004)
	Chlorothalonil	F	FR	4.7	+	Chen et al. (2001)
	Chlorothalonil	F	FR	6.5	−	Bending et al. (2007)
	Chlorothalonil	F	FR	7.2	+	Chen et al. (2001)
	Mancozeb	F	100*FR	7.5	++	Rasool and Reshi (2010)
	Mefenoxam	F	ND	7.2	−−−	Monkiedje et al. (2002)
	Metalaxyl	F	ND	7.2	−−−	Monkiedje et al. (2002)
	Pentachlorophenol	F	100*FR	5.7	−−	Cristina Diez et al. (2006)
	Prochloraz	F	2*FR	8.6	No effect	Tejada et al. (2011)
	Tebuconazole	F	FR	6.5	−	Bending et al. (2007)
	Trichlorophenol	F	500*FR	4.2	−−−	Bello et al. (2008)
	Atrazine	H	FR	ND	−−−	Sebiomo et al. (2012)
	Atrazine	H	10*FR	7.1	−	Radivojevic et al. (2008)
	Aurora 40 WG	H	40*FR	ND	No effect	Bacmaga et al. (2012)
	Butachlor	H	ND	6.1	++	Xia et al. (2011)
	Butachlor	H	4*FR	ND	+	Min et al. (2001)
	Dinoterb	H	FR	5.9	−	Beulke and Malkomes. (2001)
	Dinoterb	H	FR	6.9	−	Beulke and Malkomes. (2001)
	Diuron	H	FR	8.2	No effect	Romero et al. (2010)
	Glyphosate	H	FR	ND	−−−	Sebiomo et al. (2012)
	Glyphosate	H	10*FR	6.3	−−−	Bennicelli et al. (2009)
	Glyphosate	H	10*FR	7.5	−	Bennicelli et al. (2009)
	Glyphosate	H	10*FR	7.5	−−−	Bennicelli et al. (2009)
	Metazachlor	H	FR	5.9	−	Beulke and Malkomes. (2001)
	Metazachlor	H	FR	6.9	−	Beulke and Malkomes. (2001)
	Paraquat	H	FR	ND	−−	Sebiomo et al. (2012)
	Primextra	H	FR	ND	−−−	Sebiomo et al. (2012)
	Acetamiprid	I	100*FR	7.8	−	Yao et al. (2006)
	Chlorpyrifos	I	200*FR	6.8	−	Sharma et al. (2010)
	Chlorpyrifos	I	1000*FR	6.6	−−−	Jastrzebska. (2011)
	Endosulfan	I	100*FR	5.2	+++	Kalyani et al. (2010)
	Endosulfan	I	200*FR	4.8	No effect	Defo et al. (2011)
	Endosulfan	I	200*FR	6.4	+	Defo et al. (2011)
	Endosulfan	I	200*FR	6.8	−	Sharma et al. (2010)
	Fenamiphos	I	10*FR	6.0	No effect	Caceres et al. (2009)
	Fenamiphos	I	10*FR	6.2	No effect	Caceres et al. (2009)
	Fenamiphos	I	10*FR	6.9	No effect	Caceres et al. (2009)
	Fenamiphos	I	10*FR	8.5	No effect	Caceres et al. (2009)
	Methyl parathion	I	ND	ND	−−	Bindhya et al. (2009)
	Profenofos	I	1000*FR	ND	−−	Kalam et al. (2004)
	Quinalphos	I	ND	7.0	−	Mayanglambam et al. (2005)
	Bromoxynil + Prosulfon	H + H	100*FR	6.2	−−−	Pampulha and Oliviera. (2006)
	Dieldrin + simazine + trifluralin	I + H + H		6.1	++	Fragoeiro and Magan (2008)

Enzymes	Pesticides	Type	Pesticides doses	pH of soil	Effect of pesticide between 28 and 50 days of incubation	References
	Methyl bromide + Chloropricin	Fumigant + F	FR	7.7	−	Klose et al. (2006)
Fluoresceine di-acétate hydrolase	Dichlorophenoxyacetic acid	H	10*FR	6.6	=	Zabaloy et al. (2008)
	Glyphosate	H	10*FR	6.6	No effect	Zabaloy et al. (2008)
	Imazethapyr	H	10*FR	7.2	+	Perucci et al. (2000)
	Metsulfuron-methyl	H	10*FR	6.0	No effect	Zabaloy et al. (2008)
	Rimsulfuron	H	10*FR	7.2	No effect	Perucci et al. (2000)
	Chlorpyrifos	I	100*FR	6.7	−	Dutta et al. (2010)
	Endosulfan	I	100*FR	5.2	++	Kalyani et al. (2010)
	Ethion	I	10*FR	5.1	−	Bishnu et al. (2012)
	Novaluron	I	10*FR	7.2	No effect	Das et al. (2007)
Phosphorus cycle enzymatic activities						
Acid phosphatase	Mefenoxam	F	ND	7.2	+	Monkiedje et al. (2002)
	Metalaxyl	F	ND	7.2	+	Monkiedje et al. (2002)
	Pentachlorophenol	F	100*FR	5.7	=	Cristina Diez et al. (2006)
	Validamycin	F	32*FR	7.1	+	Qian et al. (2007)
	Aurora 40 WG	H	40*FR	ND	No effect	Bacmaga et al. (2012)
	Bromoxynil	H	5*FR	7.2	−−−	Omar and Abdel-Sater (2001)
	Imazethapyr	H	10*FR	8.2	+	Perucci et al. (2000)
	Rimsulfuron	H	10*FR	8.2	No effect	Perucci et al. (2000)
	Acetamiprid	I	50*FR	5.3	−−−	Punitha et al. (2012)
	Chlorpyrifos	I	1000*FR	6.6	−	Jastrzebska. (2011)
	Endosulfan	I	10*FR	5.2	+	Kalyani et al. (2010)
	Endosulfan	I	200*FR	4.8	−	Defo et al. (2011)
	Endosulfan	I	200*FR	6.4	No effect	Defo et al. (2011)
	Selectron	I	5*FR	7.2	−−	Omar and Abdel-Sater (2001)
	Mancozeb + Dimethomorph	F + F	100*FR	6.4	−	Cycoń et al. (2010)
	Methyl bromide + Chloropricin	Fumigant + F	FR	7.7	−	Klose et al. (2006)
Alkaline phosphatase	Mancozeb	F	200*FR	6.8	−	Sharma et al. (2010)
	Mancozeb	F	100*FR	7.5	−	Rasool and Reshi (2010)
	Mefenoxam	F	ND	7.2	−−	Monkiedje et al. (2002)
	Metalaxyl	F	ND	7.2	−	Monkiedje et al. (2002)
	Aurora 40 WG	H	40*FR	ND	No effect	Bacmaga et al. (2012)
	Bromoxynil	H	5*FR	7.2	++	Omar and Abdel-Sater (2001)
	Butachlor	H	ND	6.1	−	Xia et al. (2011)
	Imazethapyr	H	10*FR	8.2	+	Perucci et al. (2000)
	Rimsulfuron	H	10*FR	8.2	No effect	Perucci et al. (2000)
	Acetamiprid	I	50*FR	5.3	−−−	Punitha et al. (2012)
	Chlorpiryfos	I	1000*FR	6.6	−	Jastrzebska. (2011)
	Chlorpyrifos	I	200*FR	6.8	−	Sharma et al. (2010)
	Endosulfan	I	200*FR	4.8	++	Defo et al. (2011)
	Endosulfan	I	200*FR	6.4	++	Defo et al. (2011)
	Endosulfan	I	200*FR	6.8	No effect	Sharma et al. (2010)
	Quinalphos	I	ND	7.0	No effect	Mayanglambam et al. (2005)
	Selectron	I	5*FR	7.2	+	Omar and Abdel-Sater (2001)
	Mancozeb + Dimethomorph	F + F	100*FR	6.4	−	Cycoń et al. (2010)
Phosphomonoesterase	Carbendazim	F	2*FR	6.8	No effect	Yan et al. (2011)
	Chloramphenicol	F	2*FR	6.8	−−−	Yan et al. (2011)

Enzymes	Pesticides	Type	Pesticides doses	pH of soil	Effect of pesticide between 28 and 50 days of incubation	References
	Metalaxyl	F	2*FR	8.2	+	Sukul (2006)
	Prochloraz	F	2*FR	8.6	No effect	Tejada et al. (2011)
	Propiconazole	F	1000*FR		−−	Kalam et al. (2004)
	Trichlorophenol	F	500*FR	4.2	No effect	Bello et al. (2008)
	Butachlor	H	4*FR	ND	−−−	Min et al. (2001)
	Diflufenican	H	ND	6.9	−−	Tejada. (2009)
	Glyphosate	H	ND	6.9	−−	Tejada. (2009)
	Acetamiprid	I	100*FR	7.8	−	Yao et al. (2006)
	Cadusaphos	I	10*FR	7.4	No effect	Vavoulidou et al. (2009)
	Dichlorvos	I	ND		−−	Madhuri and Rangaswamy (2002)
	Methomyl	I	ND		−−	Madhuri and Rangaswamy (2002)
	Phorate	I	ND		−−	Madhuri and Rangaswamy (2002)
	Carbendazim + Chloramphenicol	F + F	2*FR	6.8	−−−	Yan et al. (2011)
	Chlorpyrifos + carbandazim	F + I	5*FR	8.8	+	Srinivasulu et al. (2012)
	Monocrotofos + Mancozeb	I + F	5*FR	8.8	+	Srinivasulu et al. (2012)
Carbon cycle enzymatic activities						
β-Glucosidase	Fluazinam	F	10*FR	6.0	No effect	Maarit Niemi et al. (2009)
	Mefenoxam	F	ND	7.2	−−	Monkiedje et al. (2002)
	Metalaxyl	F	ND	7.2	−	Monkiedje et al. (2002)
	Metalaxyl	F	2*FR	8.2	−	Sukul (2006)
	Prochloraz	F	2*FR	8.6	No effect	Tejada et al. (2011)
	Trichlorophenol	F	500*FR	4.3	No effect	Bello et al. (2008)
	Cadusaphos	I	10*FR	7.4	No effect	Vavoulidou et al. (2009)
	Endosulfan	I	200*FR	4.8	−−−	Defo et al. (2011)
	Endosulfan	I	200*FR	6.4	+	Defo et al. (2011)
	Ethion	I	10*FR	5.1	−	Bishnu et al. (2012)
	Diflufenican	H	ND	6.9	−	Tejada. (2009)
	Glyphosate	H	ND	6.9	−	Tejada. (2009)
	Linuron	H	10*FR	6.0	No effect	Maarit Niemi et al. (2009)
	Metribuzin	H	10*FR	6.0	No effect	Maarit Niemi et al. (2009)
	Methyl bromide + Chloropricin	Fumigant + F	FR	7.7	No effect	Klose et al. (2006)
Cellulase	Fluazinam	F	10*FR	6.0	No effect	Maarit Niemi et al. (2009)
	Propiconazole	F	100*FR	7.6	−	Ramudu et al. (2011)
	Propiconazole	F	100*FR	7.7	−	Ramudu et al. (2011)
	Bromoxynil	H	5*FR	7.2	−	Omar and Abdel-Sater (2001)
	Linuron	H	10*FR	6.0	No effect	Maarit Niemi et al. (2009)
	Metribuzin	H	10*FR	6.0	No effect	Maarit Niemi et al. (2009)
	Monocrotophos	I	5*FR	7.2	+	Gundi et al. (2007)
	Monocrotophos	I	5*FR	8.2	+	Gundi et al. (2007)
	Profenofos	I	ND	7.4	++	Nasreen et al. (2012)
	Endosulfan	I	ND	7.4	++	Nasreen et al. (2012)
	Quinalphos	I	5*FR	7.2	+	Gundi et al. (2007)
	Quinalphos	I	5*FR	8.2	+	Gundi et al. (2007)
	Cypermethrin	I	5*FR	7.2	++	Gundi et al. (2007)
	Cypermethrin	I	5*FR	8.2	++	Gundi et al. (2007)
	Selectron	I	5*FR	7.2	No effect	Omar and Abdel-Sater (2001)
	Dieldrin + simazine + trifluralin	I + H + H		6.1	++	Fragoeiro and Magan (2008

Enzymes	Pesticides	Type	Pesticides doses	pH of soil	Effect of pesticide between 28 and 50 days of incubation	References
Nitrogen cycle enzymatic activities						
Urease	Carbendazim	F	2*FR	6.8	+++	Yan et al. (2011)
	Chloramphenicol	F	2*FR	6.8	No effect	Yan et al. (2011)
	Mancozeb	F	100*FR	7.5	−−	Rasool and Reshi (2010)
	Metalaxyl	F	2*FR	8.2	−	Sukul (2006)
	Prochloraz	F	2*FR	8.6	No effect	Tejada et al. (2011)
	Tri-chlorophenol	F	500*FR	4.3	−−−	Bello et al. (2008)
	Validamycin	F	32*FR	7.1	+	Qian et al. (2007)
	Aurora 40 WG	H	40*FR		No effect	Bacmaga et al. (2012)
	Butachlor	H	ND	6.1	−	Xia et al. (2011)
	Diflufenican	H	ND	6.9	−−	Tejada. (2009)
	Diuron	H	FR	8.2	No effect	Romero et al. (2010)
	Glyphosate	H	ND	6.9	−	Tejada. (2009)
	Fenamiphos	I	10*FR	6.9	−	Caceres et al. (2009)
	Mancozeb + Dimethomorph	F + F	100*FR	6.4	No effect	Cycoń et al. (2010)
	Carbendazim + Chloramphenicol	F + F	2*FR	6.8	++	Yan et al. (2011)
	Deltrametrin + Propineb	I + F	ND	ND	++	Rahmansyah et al. (2009)
Sulfur cycle enzymatic activities						
Arylsulphatase	Fluazinam	F	FR	6.0	No effect	Maarit Niemi et al. (2009)
	Metalaxyl	F	2*FR	8.2	+	Sukul (2006)
	Diflufenican	H	ND	6.9	No effect	Tejada. (2009)
	Glyphosate	H	ND	6.9	No effect	Tejada. (2009)
	Metribuzin	H	FR	6.0	No effect	Maarit Niemi et al. (2009)
	Cadusaphos	I	10*FR	7.4	No effect	Vavoulidou et al. (2009)
	Endosulfan	I	100*FR	5.2	++	Kalyani et al. (2010)
	Selectron	I	5*FR	7.2	−−	Omar and Abdel-Sater (2001)
	Methyl bromide + Chloropricin	Fumigant + F	10*FR	7.7	No effect	Klose et al. (2006)

F fungicide, *H* herbicide, *I* insecticide, *FR* field rate, *ND* no mentioned

+ (increased from 5 to 40 %), ++ (increased from 40 to 70 %), +++ (higher than 70 %)

− (reduced from 5 to 40 %), −− (reduced from 40 to 70 %), −−− (reduced by 70 %)

Table 3. Overall effects of pesticides on enzymatic activities

Enzymes	No. of experiments	Percentages of response		
		Positive	No effect	Negative
Dehydrogenase	49	23	16	61
Fluorescein di-acetate hydrolase	9	22	44	34
Acid phosphatase	16	28	22	50
Alkaline phosphatase	18	28	22	50
Phosphatase	17	18	23	59
β-Glucosidase	15	6	47	47
Cellulase	16	56	25	19
Urease	16	25	31	44
Aryl-sulfatase	9	22	67	11

Fig. 1. Agricultural landscapes and microcosms

Fig. 2. Effect of pesticide action mechanisms on the overall response of enzymatic activities. F1 Affect respiration process and production of energy, F2 Phenylamides affect RNA synthesis, H1 Inhibition of acetolactate synthase, H2 Inhibitors of 5-enolpyruvylshikimate-3-phosphate synthase, H3 Inhibitors of photosynthesis at photosystem II, I1 Inhibition of the enzyme acetylcholinesterase of nerve impulses, I2 Alter the movement of ions across the nerve cell membrane