Effects of Herbicides on Fungal Phytopathogens

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Abstract – Herbicides are inevitable inputs to control excessive weed in crop land, particularly where modern agricultural practices such as conservation tillage, are opted. Intensive farming has increased the market value of herbicides among the other pesticides. Although herbicides are effective in controlling weed population, administration of this synthetic chemicals may alter the soil microbial community causing potential increase of plant pathogens. Moreover, herbicides may also have non-target effects on the cultivated crops making them more susceptible to diseases. Actions of herbicides in soil that either stimulate microbial growth or wipe out some microbial population may create space for the thrival of opportunistic fungi. Previous studies showed that white rot fungi are more tolerant to herbicides as they produce lignin degrading enzymes that are highly oxidative, non-specific and are able to transform a wide range of herbicides. Besides that, this group of fungi can grow on agricultural waste substrates. Influence of these herbicides on soil microbial ecosystem and interactions of plants and pathogenic white rot fungi modulate disease development in plant hosts.

Keywords: Herbicide, lignin degrading enzymes, soil microorganisms, white rot fungi

Introduction

Since the dawn of civilization, human have been trying to produce adequate food products to feed his kind. The application of sulphur to control insects was adapted from Sumerians back from 2500 BC, and the Chinese have used natural organic compounds as seed treatment prior to planting to reduce pest attack (Conway & Pretty, 1991). It is a major challenge to produce food to sustain the increase of human population which is predicted to be 9.8 billion by 2050 (UN, 2017). The rapid expansion of agricultural food production has been impressive over the past decades and the current value of world agricultural produce is approximately $2200 billion (FAO, 2015). The increase in agricultural crop yield per unit area in the limited arable land is largely reliant on judicious use of pesticides (Oerke, 2006). The increase in the market value of good quality agricultural produce drove the farmers to protect their crops from pests and diseases. This has led to rapid expansion of the world pesticide industry which is clearly portrayed by the increasing annual pesticide sales in major agricultural cropland regions (Carvalho, 2017).

Pesticide is a combination of the English word ‘pest’ and the Latin word ‘cida’ which means ‘to-kill pest’ (Singh, 2012). Pest is described as any unwanted organism which is noxious, nuisance and destructive (Hassall, 1982). Pests can range from weeds, fungi, bacteria, viruses, and insects; several vertebrates such as rats and birds and lower animals (invertebrates) including nematodes and parasitic worms are also important crop pests (Cremlyn, 1990). The production and commercialization of pesticide began after the war in 1945, when the selective broadleaf herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) and 2-methyl-4-chlorophenoxyacetic acid (MCPA) were introduced (Cobb & Kirkwood, 2000). This was followed by the introduction of organophosphates,
carbamates, synthetic pyrethroids, modern herbicides, fungicides and lately the neonicotinoids (Pretty & Bharucha, 2015).

Various types of pesticides were discovered under different classes, each effective against specific pests. The three major classes of pesticides are the insecticides, herbicides and fungicides (Cremlyn, 1990). Herbicides that are used to kill or suppress the growth of undesired plants and vegetation (Cork & Kreuger, 1992) account for the largest market among pesticides followed by insecticides and fungicides (Moreira et al., 2013). In 1970s, the herbicide market value was US$3 billion with 6.3% growth per annum (Carvalho, 2017). In the subsequent decade, the market growth slowed down to 4.5% per annum and a static growth of 0.1% was reported in the 1990s with a market value of US$ 31 billion (Cobb & Kirkwood, 2000).

Weeds are noxious unwanted plants that cause significant loss to the farmers. Oerke (2006) stated that 34% of reduction in crop production is caused by weeds while insect pests and microbial pathogens cause 18% and 16% of crop product loss respectively. Earlier, mechanical tillage method was used to manage the weed population in crop lands. However, this practice affected the soil health as it increased the risk of soil erosion and decreased soil organic matter content which eventually affected the crop cultivation (Six et al., 1999). The “conservation tillage” and “no-tillage” practices which were used to maintain the soil health have led to higher reliance on synthetic chemicals to control weed (Rose et al., 2016).

Mode of Action of Herbicides
Herbicides have been a convenient, economical, effective and immediate solution in weed management in agricultural lands (Montanya et al., 2013). The earliest form of herbicides include crude chemicals such as sulphuric acid, arsenic ores, rock salt and copper salt that were administrated during mass clearing of land to kill all the plants (Hassall, 1982). But, these chemicals were non-selective. Over the past 50 years, various research and development programmes have generated hundreds of herbicidal active ingredients and many were already commercialized (Duke, 2012). These herbicides are highly selective to plants, less toxic to non-target organisms, effective even in low dosage, less harmful to environment, and have lower production cost.

For efficient weed control, it is important to identify and select appropriate herbicides. It is also necessary to have basic information on the factors that affect the efficiency of the herbicides prior to application. Herbicides can be applied post-emergence or pre-emergence of weeds (Wagner, 2006). Administration of herbicides post-emergence of weed should take into count the weed species present in a particular area, the size of the weed plant, population density, growth rate and environmental conditions, whereas for the pre-emergence type of application, the soil type and soil condition have to be considered (Montanya, 2013). The mechanism of herbicide action comprises of a few processes before causing plant death. First of all, herbicides need to be applied in sufficient dosage and must be absorbed by the weed. Next, the absorbed chemical should move to the target action site in weed, affecting its physiology leading to death (Sherwani et al., 2015).

The most widely used herbicide in the world is glyphosate (Coupe et al., 2012). Glyphosate N-(phosphonomethyl)glycine, is the active ingredient in a non-selective systemic herbicide used worldwide under the commercial name, Roundup (Woodburn, 2000). After this chemically weak acid is absorbed through foliar tissues, it is translocated by phloem tissues to the apical meristematic regions. Glyphosate uptake through root rarely occurs as glyphosate that binds to soil particles becomes inactive and is irreversible (Macdonald et al., 2013). Glyphosate inhibits the 5-enolpyruvylshikimate synthase (EPSP) in the shikimate pathway of the specific target organism through chelation of Mn, which is a cofactor for EPSP synthesis (Senseman, 2007). Glyphosate can inhibit EPSPs in not only weeds but also in fungi, and bacteria (Kishore & Shah 1988).

Glufosinate and paraquat are non-selective contact herbicides that are commonly used to control a wide range of weeds. They need to be applied on foliage to be absorbed by the weed and impair the target site. Both of these herbicides are inactive in soil due to irreversible binding with soil particles.
Glufosinate and paraquat only cause injury in the treated foliage region, therefore are more effective in controlling annual weeds compared to perennial plants.

Glufosinate absorbed by leaf inhibits the glutamine synthase in chloroplast, which catalyzes ammonia into glutamate to form glutamine (Shelp et al., 1992). This step is crucial for the plant to obtain nitrogen for cellular activities. When glutamine synthase is inhibited, free ammonia level in the chloroplast increases uncoupling of membranes. Hence, the membranes will not be able to maintain energy formation gradient during photosynthesis. The weed dies as they are not able to perform photosynthesis (Wild et al., 1987).

Similarly, paraquat is also absorbed through leaves and is active in chloroplast. Members of bipyridylium such as paquat and diquat affects the electron transport in photosystem. Paraquat can affect photosystem I during photosynthesis (Devine et al., 1993), by impeding the mitochondrial function, where the electron transfer from ferredoxin to ferredoxin: nicotinamide adenine dinucleotide phosphate oxidoreductase (FNR) is disrupted (Funderburk & Lawrence, 1964). The electron from ferredoxin Fp/Fr is used by paraquat to produce oxidized paraquat and oxygen radicals. These oxygen radicals react with the chloroplast and also the lipid fraction of membranes (Vaughn & Duke, 1983). Hence, plants are not able to undergo photosynthesis and die.

### Roles of Herbicides in Plant Disease Development

Herbicides do not only affect the weed population but are able to predispose plants to diseases under certain conditions (Altman & Campbell, 1977). Frequent application of herbicides in conventional farms has raised several issues associated with the interaction of herbicides with plant nutrients, development of resistant-weeds and also harmful effects on soil microflora that may cause changes in plant disease expression (Johal & Huber, 2009). The change in micronutrient availability can adversely affect plant physiological functions altering the growth and resistance towards pest and diseases (Datnoff et al., 2007).

Glyphosate inhibits EPSP synthase due to overlapping in binding sites of phosphoenolpyruvate (PEP) and glyphosate on the enzyme (Tan et al., 2006). Competitive binding of glyphosate to the allosteric site of EPSP synthase alters the protein structure, preventing PEP binding and disrupting the antimicrobial phytoalexin productions in plant (Dill, 2005). It also starves plants from aromatic amino acids (AAR) - tryptophan, phenylalanine, tyrosine and secondary products which are essential in plant physiological processes, increasing the risk of disease development in plants (Rahe et al., 1990; Tan et al., 2006, Gomes, 2014). The biosynthesis of salicylic acid (SA) which is a key component of plant systemic resistance is affected when glyphosate inhibits the conversion of shikimate to chorismate in the shikimate pathway as SA is directly synthesized from chorismic acid (Johal & Huber, 2009). Therefore, glyphosate may be beneficial to several phytopathogens such as Magnaporthe, Gaeumannomyces and Corynespora (Thompson & Huber, 2007). EPSPs are also found in a few classes of phytopathogenic fungi including basidiomycetes, ascomycetes and fungal deuteromycetes (Dill, 2010). Thus, glyphosate may have antifungal activity on Cercospora, Peronospora and Erisiphe by inhibiting their EPSPs thus suppressing disease development in crop plants (Nandula, 2010).

Nitrogen (N) is one of the basic nutrients needed for plant growth and disease resistance. Lack of N can lead to weaker plants and increase susceptibility to diseases. The inorganic N in atmosphere is reduced to ammonia before being incorporated into glutamine, glutamate, asparagine and aspartate, which are N-containing molecules in plants (Lam et al., 1995). These molecules are predominant in the synthesis of amino acids, proteins, hormones and chlorophyll (Crawford, 1995). Phytopathogens acquire N from host tissue for successful colonization. The N sources available for the pathogens differ depending on the type of tissues being attacked. The N preference for root pathogens may differ from those of foliar pathogens. Although less information is available on the N source metabolism by the phytopathogenic fungi after entering the host plant, it is speculated that N limitation affects the development of phytopathogen in host (Snoeijers et al., 2000). N limitation by application of glufosinate may affect glutamine synthase of cultivated crops, reducing N available to plants. Agrios
(2005) reported that the lack of N increased susceptibility of tomato to Fusarium wilt, Alternaria blight in solanaceous crops and Pythium infection in seedlings, whereas a high level of N has been found to reduce Xanthomonas colonization in cabbage and development of black rot lesions. Thus, the lack of N in plant after glufosinate treatment might be one of the in planta environmental factors that is able to induce the expression of avirulent or virulent genes involved in pathogenesis. Additionally, transgenic rice plants that are tolerant against herbicide glufosinate have shown enhanced disease resistance towards rice blast and sheath blight caused by Magnaporthe oryzae and Rhizoctonia solani (Ahn, 2008). Mycelial growth of R. solani was found to be inhibited by glyphosate, glufosinate ammonium and paraquat (Black et al., 1996). Paraquat prevented the sclerotia formation, whereas glufosinate reduced only the count of colony forming unit (Black et al., 1996). Thus, the effects of herbicides on soil microbes that lead to disease development are complex and influenced by various factors.

**Effects of Herbicides on Soil Microbial Population**

Soil microbes play an important role in many soil processes including breakdown of organic matter, release and availability of nutrients in soil and degradation of xenobiotics (Bending, 2002). The microbial population in soil can be efficient indicators of soil health and environmental changes (Avidano et al., 2005). The non-target toxicity effects of herbicides include the alteration of microbial population structure, function and soil health (Zabaloy, 2008). Araújo (2003) found that administration of glyphosate on soil may exert at least temporary changes in the soil microbial activity. The number of heterotrophic bacteria increased in soil with glyphosate residue and a higher fungal count were found in soil when supplemented with a low dosage of herbicide for the first time. Application of glyphosate also increased the aerobic heterotrophic bacterial (AHB) density and substrate-induced respiration (SIR) in soil as the organophosphonate glyphosate can be a source of phosphorus, carbon and N for microbes (van Eerd et al., 2003). Glyphosate application at a rate of 50 mg kg⁻¹ was found to increase the fungal population in soil (Ratcliff, 2006). Lancaster et al. (2010) found that microbial population in the soil adapt to the herbicide content and starts utilizing the glyphosate as a nutrient source after repeated application.

Previous study demonstrated that repeated application of paraquat or diquat in plots with conventional tillage reduced the number of microorganisms especially the common saprophytic fungi in soil (Rose et al., 2016). Smith and Lyon (1976) found that the pathogenic Mucor and Zygorhynchus were more susceptible to paraquat exposure compared to Aspergillus and Penicillium. The increasing level of paraquat in media inhibited the hyphal growth in Mucor and Zygorhynchus compared to Aspergillus and Penicillium. The dry matter of Mucor and Zygorhynchus was reduced by 50% even with 130 mg/L of paraquat, whereas the same reduction level in Aspergillus and Penicillium needed a higher concentration of paraquat. In contrast, Zain et al. (2013) found that a higher concentration of paraquat and glufosinate ammonium caused severe growth inhibition (70%-100%) in Mucor, Aspergillus and Penicillium. However, glyphosate was found to be less toxic to Aspergillus and Penicillium while causing inhibitory effect on Mucor.

The N starvation-stress caused by glufosinate is known to affect a number of morphogenetic processes in microbes. In Saccharomyces cerevisiae, N limitation causes a pseudohyphal, invasive growth pattern (Gimeno et al., 1992), whereas for A. nidulans, carbon and N starvation was found to induce BrlA, the central regulator of sporulation (Skromme et al., 1995). Besides that, very few other fungal species belonging to Ascomycete group was found to be inhibited by paraquat and glufosinate ammonium at low concentration (Bromilow, 2004).

**Herbicide Effects on White Rot Fungi (WRF) and Degradation by Lignolytic Enzymes**

White rot fungi (WRF) are filamentous Basidiomycetes known to degrade lignin and all lignocellulose components (Pointing, 2001). Oxidative bleaching and lignin degradation of plant by WRF leave behind fibrous texture which is pale in colour. The degradation process of WRF is based on a non-specific, radical based mechanism with the aid of extracellular enzymes secreted by the fungi, mainly laccase, lignin peroxidase and manganese peroxidase (Maciel et al., 2012). These
enzymes are collectively known as lignin-modifying enzymes (LMEs). LMEs are highly non-specific thus react with a wide range of compounds that have similar structure to lignin (Kersten & Cullen, 2007). There are a few reviews on the LMEs produced by WRF including their mechanisms and functions in substance degradation (Moreira et al., 2013). These LMEs function separately or cooperatively with the aid of auxiliary enzymes that generate hydrogen peroxides, dehydrogenases and also hydroxyl radicals (●OH). Chelated manganese (III) (Mn^{3+}) ions are also necessary to complete the lignin or xenobiotic degradation (Hammel et al., 2002; Hofrichter, 2002).

The worldwide distribution of WRF and their ability to degrade herbicides have made them interesting microorganisms to be studied. In the past decade alone, various studies have been conducted on degradation of various environmental pollutants including herbicides by WRF or ligninolytic fungi (Asgher et al., 2008). *Phanerochaete chrysosporium* is one of the widely used WRF in degradation of herbicides (Singh and Singh, 2016; Fragoeiro and Magan, 2005; Muogin et al., 1994). The other genera of WRF that are found to be tolerant to herbicides with high potential in transforming them include *Trametes*, *Coriolus* and *Pleurotus* (Asgher et al., 2008). *Ganoderma* particularly *G. lucidum* was also found to be resistant towards certain classes of herbicides and induce the production of laccase (Coelho et al., 2010).

The degradation or transformation of herbicides by WRF may follow two proposed systems. The first system involves LMEs and takes place in the extracellular region of the fungi (Moreira et al., 2013). The non-specific LMEs can act on the chemical molecules, and in some cases the process is enhanced by mediators. Mediators can enhance the enzymatic reactivity of LMEs on substrate. This was shown in recent study, where glyphosate was transformed into aminomethyl phosphonic acid (AMPA) in *in vitro* cultures added with purified LMEs in combination with different mediators such as hydrogen peroxide, MnSO₄, Tween 80 and veratryl alcohol (Pizzul et al., 2009). AMPA is commonly found in soils treated with glyphosate although the degradation rate seems slower (Borggaard, 2008), therefore the transformation of glyphosate in agricultural soils can also be explained by the LMEs from WRF found in soil. Moreover, due to their non-specificity, the herbicide transformation is not only limited to glyphosate. In Pizzul’s study (2009), 70-100% of a mixture of 22 herbicides were degraded by manganese peroxidase in the presence of Tween 80 and MnSO₄. Herbicide bentazon was efficiently transformed in 24 hours by laccase from *G. lucidum* added with 2,2’-azino-bis,3-ethylbenzothiazoline-6-sulphonic acid (ABTS), hydrogen peroxide and Tween 80 (Coelho et al., 2010).

The second WRF herbicide degradation system occurs intracellular, mainly involving cytochrome P450 (Moreira et al., 2017; Koroleva et al., 2015; Coelho et al., 2010). When cytochrome P450 is present, the ionization potential of environmentally persistent aromatics are lower, thus providing suitable substrates for ligninolytic one-electron oxidizing enzymes for effective degradation (Hiratsuka et al., 2001). The addition of microsomal fractions from *Pleurotus ostreatus* was able to transform the herbicide in the presence of NADPH suggesting the involvement of cytochrome P450 (Jauregui, 2003). In addition, the *P. chrysosporium* was able to detoxify herbicide through the P450 monoxygenase system (Matsuzaki & Wariishi, 2004). The study on the generic ability of WRF to degrade herbicide based on their ligninolytic properties showed that WRF are able to degrade various groups of herbicide but the mechanism involved could not be distinguished (Bending et al., 2002).

Conclusions

WRF from a variety of basidiomycete orders have been explored for their potential in herbicide and pollutant biodegradation and transformation properties. Application of common herbicides especially glyphosate, paraquat and glufosinate may cause growth stimulation or inhibition of various fungal species but less is known about the tolerance of WRF exposed to herbicides as it depends on the dosage and frequency of application. Therefore, further research on the WRF tolerance to herbicides in relation to the phytopathogenicity is very crucial to overcome disease development and to enhance crop protection.
References


