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In vitro Studies on the Effects of Sugar Beet Fields Prevalent Herbicides on the Beneficial and Deletrious Fungal Species

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Abstract: The antifungal activities of five herbicides prevalent in sugar beet fields were investigated qualitatively *In vitro* in the case of fungal species including *Bipolaris* sp., *Ceratocystis radicicola*, *Fusarium culmorum*, *F. graminearum*, *F. proliferatum*, *F. oxysporum*, *Gaeumannomyces graminis*, *Macrophomina phaseolina*, *Phytophthora* sp., *Pythium* sp., *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Talaromyces flavus*, *Trichoderma koningii*, *T. longibrachiatum*, *T. hamatum*, *T. harzianum*, *Trichoderma* sp., and *Verticillium dahliae*. 2,4-D, desmedipham and diclofop-methyl showed the most broad antifungal effects and clodinafop and tralkoxydium had a similar ranges narrower than of other herbicides tested. No oomycetous fungus (*Phytophthora* and *Pythium*) was able to grow on the toxic media. This is the first global report on the antifungal effects of the herbicides, diclofop-methyl (Illoxanr®) and clodinafop (Topik®).

Key words: Fungus, herbicide, sugar beet, ecotoxicology, Iran

Introduction

The control of soilborn fungi is difficult in soil and although soil strilization by the fumigants such as methyl bromide, metham sodium and others is very effective, but not economically feasible in fields. The best method to control such diseases is the application of resistant varieties, but this way is too time-consuming to be applied practically sooner.

Therefore, improved cultural practices together with biocontrol measures may be useful to control such diseases. Such an integrated disease management needs much information about the various ecological and biological aspects that this work may be a more step on the line of such an illustration.

Sclerotinia sclerotiorum (Lib.) de Bary, as an important plant pathogen causes substantial losses in crop production throughout the world each year (Purdy, 1979). Diseases caused by this pathogen occur in numerous plant hosts. The broad range of this fungus is important to the control of the disease in agricultural crops because it restricts the number of non-host crops that can be included in the crop rotations designed to reduce the concentration of sclerotia in infested soils. Little information is available on the relative importance of these sources of inoculum to outbreaks of the disease (Abawi and Grogan, 1975).

Based on an index of host plants of *Sclerotinia sclerotiorum* compiled by Boland and Hall (1994), the fungus attacks to 42 subspecies or varieties, 408 species, 278 genera and 75 families. Usage of herbicides may cause the disease limitation through eradication of host weeds, and also may have direct harmful effects on the growth and development of the disease.

Materials and Methods

The fungi studied were obtained from the Division of Plant Diseases Research of the Institute of Plant Pests and Diseases Research, Evin, Tehran and Department of Plant Pathology, Faculty of Agriculture, Tarbiat Modarres University, Tehran.

Bipolaris sp., *Ceratocystis radicicola*, *Fusarium culmorum*, *F. graminearum*, *F. proliferatum*, *F. oxysporum*, *Gaeumannomyces graminis*, *Macrophomina phaseolina*, *Phytophthora* sp., *Pythium* sp., *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Talaromyces flavus*, *Trichoderma Koningii*, *T. longibrachiatum*, *T. hamatum*, *T. harzianum*, *Trichoderma* sp., and *Verticillium dahliae*.

The herbicides prevalent in sugar beet field applications were studied including desmedipham (Betanal® A-M, EC 15.7% w/v), clodinafop (Topik®, EC 8% w/v), and tralkoxydium (Grasp® EC 10% w/v).

To study the effects of these herbicides on the fungal development, a medium based on PDA was prepared, and the above-mentioned herbicides were added to the media, so that a medium containing 5000 ppm of each herbicide was made and added in 20ml volumes to petri plates. Control media were herbicide-free. Three plates were considered for each treatment, and stored at 25°C, in dark for 20 days before the beginning of the experiment (based on pretests illustrated the unability of *Trichoderma* species to grow earlier on the media).

The same-aged fungal cultures on PDA (7 days) incubated at 25°C and in dark were used as sources of inocula. In each plate, four 5mm circular plots of four different fungi were cultured and incubated under said conditions for 7 days.

Then after, the plates were observed and the growth of fungi was detected qualitatively.

Results and Discussion

Observations made after 7 days of incubation revealed that all of the chemicals effectively inhibited the growth of *Gaeumannomyces graminis*, and the oomycetous species of *Phytophthora* and *Pythium*. In contrast, *Trichoderma harzianum*, *T. koningii* and to some extent, *T. longibrachiatum* were able to grow on all media amended with different herbicides. Behaviour of all fungi is shown in Table 1.

Table 1: Behaviour of various fungi in reaction to the herbicides tested.

Fungi	Desmedi-pham	Clodina fop	2,4-D	Diclofop-methyl	Tralkoxydium
Bipolaris sp.	-	+	+	-	-
Ceratocystis radicicola	-	-	-	-	+
Fusarium culmorum	+	+	+	+	+
F. graminearum	-	+	-	+	+
F. proliferatum	+	+	+	+	+
F. oxysporum	+	+	-	+	+
Gaeumannomyces graminis	-	-	-	-	-
Macrophomina phaseolina	-	+	-	-	+
Phytophthora sp.	-	-	-	-	-
Pythium sp.	-	-	-	-	-
Rhizoctonia solani	-	-	-	+	+
Sclerotinia sclerotiorum	-	-	-	-	+
Talaromyces flavus	+	±	-	+	+
T. koningii	+	+	±	+	+
Trichoderma Longibrachiatum	+	+	+	+	+
T. hamatum	+	+	+	-	-
T. harzianum	+	+	+	+	+
T. viride	±	+	+	-	-
Verticillium dahliae	-	-	-	+	-

+ Any form of mycelial growth observed, - No mycelial growth observed, ± mycelial growth observed only in some replicates

From the results it is clear that 2, 4-D and desmedipham have the most broad ranges of antifungal activity. 2, 4-D belongs to

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phenoxy acetic acid (auxin) group and the other is from bis-carbamate (phenyl carbamate) group (Razavi, 1995). It is interesting to remind of dithiocarbamate and ethylene-bis-dithiocarbamate fungicides, which are all derivatives of dithiocarbamic acid and as believed, toxic to fungi mainly because of their metabolization to the isothiocyanate radical (- N = C = S). This radical inactivates the sulfhydryl groups (-SH) in amino acids and in enzymes within pathogen cells and thereby inhibits the production and function of these compounds (Agrios, 1997).

Diclofop-methyl from aryloxy phenoxy propionic acid group was inhibitor for 9 fungal species out of 19 species tested. It seems that this chemical inflicts it's deletrious antifungal effects through damage caused in cell membane (Razavi, 1995), the most common and similar partition of both plant and fungal cells.

With small differences, although of different herbicidal groups, tralkoxydium and clodinafop propargyl had similar activities and anti-fungal ranges. The first is from oxims and the other is from the same group including diclofop-methyl. They may infuence on the fungal development through the effects related to respectively cell division, and lipids biosynthesis prevention, as they act in planta.

The results are of potentially valuable importance that may be practically useful in the integrated control of plant diseases. Weeds are competants for water and nutrient supplies, light and space, and in addition to their direct yield reducing effects, they are usually hosts for some of pathogens and pests. They serve pathogens as sites for over-wintering, reproduction, saprophytic survival and green bridges of importance for disease development. Therefore, they may cause a bad consequence in spite of cultural practices like crop rotation. Knowing the history of the field, The best herbicide can be chosed which effective on the pathogenic fungi of current and future importance. Results related to the effects on fungi biological control importance may be useful from the ecotoxicological and integrated control scheduling stand point.

The effect of 7 herbicides on the sporulation capacity of the aphid pathogenic fungus, *Erynia neoaphidis* investigated in laboratory had shown that phenmedipham (Betanal®, EC) was intermediate in effect (Keller and Schweizer, 1991). Other experiment performed by Kharchenko and Shklyar (1975), demonstrated that out of 10 herbicides tested, 2, 4-D was one of the four herbicides showed the highest antifungal activity against various fungal species including *Fusarium* spp., even *Fusarium sporotrichiella* (*F. sporotrichoedes*) which was resistant to all preparations except 2, 4-D. In an experiment with 25 species of beneficial organisms including 3 entomogenous fungi, tralkoxydium (Grasp®) was harmless to nearly all the beneficial organisms (Hassan *et al.*, 1994).

Based on the results of this experiment, phenmedipham had an genostatic in fluence on *Trichoderma harzianum, T. hamatum, T. koningii, T. longibrachiatum* and *Talaromyces flavus* but not on *Trichoderma* spp. Also, clodinafop had a similar effect on sporulation of the above-mentioned species. Although, *Trichoderma harzianum* had the most rapid growth as and compared to other species of the genus and *Talaromyces flavus* (with no growth), it did not sporulate on the media contained 2, 4-D.

T. harzianum did not sporulate on the media contained tralkoxydium in contrast to *Talaromyces flavus* and on the media containing diclofop-methyl, *T. harzianum* had the most aboundant sporulation followed by *T. koningii* and *T. longibrachiatum* respectively.

Macrophmina phaseolina although able to grow on the media contained tralkoxydium or clodinafop, did not produced micro-sclerotia on the media contained the first herbicide, but produced them on the media with the second chemical added.

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