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Biological control studies on *Convolvulus arvensis* L. with fungal pathogens

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ABSTRACT

Field bindweed (*Convolvulus arvensis* L.) is a perennial, noxious weed in Europe and in many agricultural areas of the world, including Turkey. Some pathogenic fungi were identified as potential to control bindweed and some of them could be used as mycoherbicide components. In the summer of 2008, 2009 and 2010 the diseased bindweed plants were collected from different sites of Amasya, Ankara, Çorum, Samsun and Tokat provinces. Pathogenic fungi were isolated from diseased plants and they were identified based on their morphological characteristics. Bindweed plants were grown in a climatically controlled room to the 4 to 5-leaf stage; they were inoculated with an aqueous spore suspension of each fungi at various densities specified. Spores were sprayed onto bindweed plants with a hand sprayer until runoff. Dates were recorded for each isolate when disease lesions became visible, and the proportions of diseased leaves, out of the total number of leaves, on each inoculated plant were recorded. *Stagonospora convolvuli, Colletotrichum linicola* and *Myrothecium verrucaria* produced the highest level of diseases on the inoculated test plants. Plant heights of *C. arvensis* were recorded the shortest following inoculation with *C. linicola* and a *Phoma* sp. These results indicate that, *C. linicola* seems potentially effective and field tests alone or in combination with *S. convolvuli,* should be performed.

Key words: mycoherbicide, field bindweed, biological control, fungi

INTRODUCTION

Field bindweed (*Convolvulus arvensis* L.) is a perennial, noxious weed in Europe and in many agricultural areas of the world, including Turkey. It is a serious problem in wheat, maize, vineyards, beans and other vegetables grown in fields. Since this weed is a deep-rooted perennial, it can survive chemical and mechanical control measures. Some control can be achieved with herbicides such as 2,4 -D, dicamba, picloram and imazapyr glyphosate. However, once a bindweed population is established it is very difficult to control. Repeated applications of herbicide may stop shoot growth and reduce the amount of root, but even after applications for several years, some roots grow, from which further shoots can develop (Timmons, 1949). Bindweed produces numerous seeds, up to 10^7 per ha, which survive for 20-30 years in the soil (Timmons, 1949). Because of these control difficulties scientists have been moving towards biological control methods since the 1970s.

Some pathogenic fungi have been identified with potential to control bindweed and be used as mycoherbicide components. The European COST Action 816 project, a five year collaboration between scientists from five European countries, made important contributions to biological control of both field and hedge bindweeds (*C. arvensis* and *Calystegia sepium*). Pfirter et al. (1997) obtained 154 fungal isolates from 28 fungus genera from bindweeds. Of these, *Stagonospora convolvuli,* strain LA39, was shown to have great potential as a bioherbicide for control of field and hedge bindweeds (Défago et al., 2001). This strain was extensively tested for effectiveness and host specificity in field trials in different locations and was found to be very effective and environmentally safe.

Phoma exigua from the south of England was found to be sufficiently effective to be considered as a potential mycoherbicide. In laboratory experiments each strain of *P. exigua* was shown to kill seedlings when applied to the three to five leaf stages with 10^6 conidia/ml. There was no regrowth from the roots (Pfirter, et al., 1997). *Phoma proboscis* was also found very effective against bindweeds in the USA (Heiny and Templeton, 1991). One of *Colletotrichum linicola* isolate (06-21) was found a destructive pathogen on field bindweeds in a climatic room experiment in Turkey (Tunali et al., 2008). In controlled environments, *Phomopsis convolvulus*, a fungus being examined as a bioherbicide for bindweed, produced 95% reduction in foliage biomass and up to 55% mortality on bindweed (Morin et al., 1989). This fungus was newly discovered in Canada (Ormeno-Nunez et al., 1988) and has since been patented as a potential biological control agent.

The objective of this study was to collect and evaluate fungus pathogens of *C. arvensis* from Turkey with the goal of ultimately producing a mycoherbicide for control of this weed.

MATERIALS AND METHODS

In the summers of 2008, 2009 and 2010 diseased bindweed plants were collected from different regions of Amasya, Ankara, Çorum, Samsun and Tokat provinces. Pathogen isolations from diseased plants were performed either by directly transferring surface-disinfested diseased tissue onto moist filter paper or by transferring diseased tissue onto half strength Potato Dextrose Agar (PDA- Merck) plates. Fungi from these isolations were identified based on their morphological characteristics. Single spore isolates of each fungus were prepared in water agar. All fungal cultures were stored in glass tubes at 4°C in the refrigerator and in 2 ml cryovials in a -85°C deep-freezer. For inoculum preparation, all fungal isolates were grown on half strength PDA (19 g potato dextrose and 10 g bacto agar in 1L distilled water). Cultures were incubated at 23±°C in plastic Petri plates from 5 to 15 days, depending on speed of growth of the fungus species. These plates were then flooded and repeatedly flushed with 10 ml sterile distilled water and spores were brushed off and collected. Spore density was determined with a haemocytometer. In total, 13 isolates used in pathogenicity tests.

C. arvensis seeds were washed under running tap water and seeds were sown in 7 cm diameter plastic pots which were transferred to a controlled environmental room. Each treatment consisted of 4 replicates, one plant in a single pot. When bindweed plants were at the 4- to 5-leaf stage, plants were inoculated with an aqueous spore suspension with 0.1% (v/v) Tween 20 (Sigma) at the various densities specified for each fungus (Table 1).

Table 1. Fungal isolates and spore concentrations used in pathogenicity tests

Fungus species	**Isolate number**	**Spores/ml**
Phomopsis convolvuli	4–9	$1x10^{6}$
Myrothecium verrucaria	25–9	$1x10^{7}$
Ascochyta sp.	26–8	$1x10^{7}$
Ascochyta sp.	10–14	$1x10^{7}$
Ascochyta sp.	10–75	$1x10^{7}$
Diplodia sp.	28–1	$5x10^{4}$
Diplodia sp.	10–08	$1x10^{7}$
Colletotrichum linicola	43–4	$1x10^{7}$
Colletotrichum linicola	9–28	$1x10^{7}$
Bipolaris sp.	10–20	$3x10^{4}$
Phoma sp.	34–1	$1x10^{7}$
Stagonospora convolvuli	9–21	$2x10^{6}$
Gloeosporium sp.	10–39	$1x10^{7}$

Spores were sprayed onto bindweed plants with a small hand sprayer until runoff. Distilled water, without spores, was applied to control plants. Pots were then covered with plastic bags for 48h. Dates were recorded for each isolate when disease lesions became visible. Pathogens were re-isolated from all diseased plants at the end of the tests. Other data included the total number of diseased and dead leaves on each diseased plant and plant height were also collected.

Data were analysed with SAS V7 software and an analysis of covariance model where fungus species was a classification variable and spore concentration a continuous covariate. This enabled adjustment of species means for spore concentration.

RESULTS AND DISCUSSION

As a result of inoculation of *C. arvensis* with these and other fungi collected in this study, significant differences were observed for proportion diseased leaves and plant heights (Table 2 and 3). The most diseased leaves were produced by inoculation with *Stagonospora convolvuli, Colletotrichum linicola,* and *Myrothecium verrucaria* (Fig. 2) and (Table 2). Diseases symptoms on *C. arvensis* caused by some of the fungi collected in this study are shown in Figure 1.

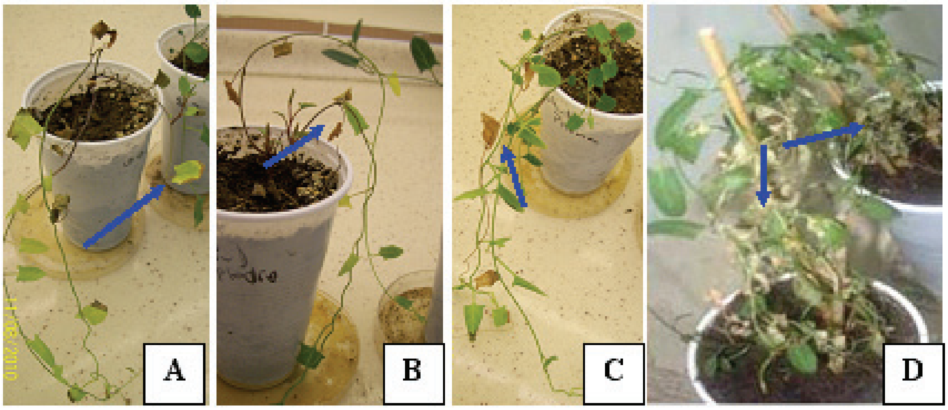

Figure 1. Symptoms on *C. arvensis* leaves caused by (A) *Ascochyta* sp., (B) *Diplodia* sp., (C) *Phoma* sp., (D) *M. verrucaria*

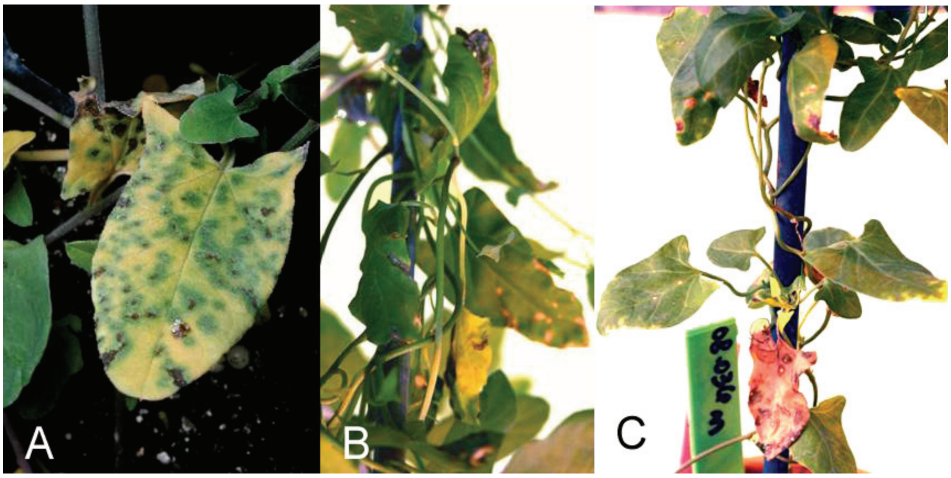

Figure 2. Symptoms on *C. arvensis* leaves. (A) *Stagonospora convolvuli* . (B) *P. convolvuli*. (C) C. *linicola*

Table 2. Mean proportion diseased and dead leaves on *C. arvensis* after inoculation with spores of fungus species. The means are adjusted, through analysis of covariance, for the covariate of spore concentration, and the probabilities associated with t-tests comparing the means to zero are indicated (P>|t|).

Fungus species	**Proportion diseased leaves**	**P>|t|**[1]
Phomopsis convolvuli	0.09	NS[2]
Myrothecium verrucaria	0.33	0.005
Ascochyta sp.	0.10	NS
Diplodia sp.	0.17	0.031
Colletotrichum linicola	0.38	<0.001
Bipolaris sp.	0.22	NS
Phoma sp.	0.20	NS
Stagonospora convolvuli	0.56	<0.001
Gloeosporium sp.	0.04	NS
Contrasts		
C. linicola minus *S. convolvuli*	-0.18	NS
C. linicola + *S. Convolvuli* minus all others	0.30	0.0001
C. linicola minus all others except *S. convolvuli*	0.22	0.03
S. convolvuli minus all others except *C. linicola*	0.39	0.003

[1] Probability of a greater absolute value of t in t-tests comparing the means to zero, i.e., the probability that the mean or contrast is greater than zero

[2] Not significantly different from zero at $P \leq 0.05$

Although a potentially effective control agent, *M. verrucaria* produces macrocyclic trichothecene that are toxins harmful to humans (Millhollon et al., 2003), this fungus was not evaluated further as a potential mycoherbicide component. Both *S. convolvuli* and *C. linicola* caused significantly more disease symptoms on leaves than all of the other fungi and there was no significant difference between these two fungi (Table 2). Surprisingly, because of the putative potential of *P. convolvuli* (Morin et al., 1989; Ormeno-Nunez et al., 1988; Kuleci 2009), the isolate of this fungus from Turkey did not produce significant proportion of diseased leaves nor significant reduction in plant height (Table 3). Plant heights of *C. arvensis* were the shortest following inoculation with *C. linicola* and a *Phoma* sp. (Table 3).

However, because of the variability in the non-inoculated water control (Table 3), these plant heights were not significantly different than the control. Inoculation with *S. convolvuli* had no effect on reducing plant height. Despite this, *S. convolvuli* has been extensively investigated and proven effective as a biological control agent of *C. arvensis* (Pfirter, et al., 1997; Défago et al., 2001). However, based on the results from this study, *C. linicola* seems at least as potentially effective and field tests with both fungi, alone and in combination, are planned. The results in Table 2 suggest that the combination of both fungi might be very effective. Of course, the effectiveness of a biological control agent can be increased by formulation which should be designed to increase both the efficiency of application and efficacy of the control agent. Different formulations of both of these fungi remain to be tested as the combination with sub-lethal dosages of herbicides. Immediate future research on these fungi will be on conidia maturity, survivability formulation dose response pathogen mixtures and susceptibility of different ecotypes of *C. arvensis*.

Table 3. Mean *C. arvensis* plant heights after inoculation with spores of fungus species. The means are adjusted, through analysis of covariance, for the covariate of spore concentration.

Fungus species	Plant height (cm)	Standard error
Phomopsis convolvuli	33.5 A*	4.54
Myrothecium verrucaria	26.4 AB	3.68
Ascochyta sp.	28.7 A	2.41
Diplodia sp.	31.5 AC	2.63
Colletotrichum linicola	21.4 B	2.78
Bipolaris sp.	31.4 AB	5.94
Phoma sp.	21.6 BC	3.68
Stagonospora convolvuli	33.8 A	4.24
Gloeosporium sp.	26.4 AB	3.68
Non-inoculated control (water)	31.7 AB	5.95

*Means followed by the same letter are not significantly ($P \leq 0.05$) different

ÖZET

FUNGAL PATOJENLER ILE *CONVOLVULUS ARVENSIS* L.' İN BİYOLOJİK MÜCADELESİ ÇALIŞMALARI

Tarla sarmaşığı (*Convolvulus arvensis*) çok yıllık zararlı bir yabancı ot olup gerek Avrupa'da gerekse Türkiye'de pek çok tarım alanında bulunmaktadır. Tarla sarmaşığıyla mücadelede potansiyel bir mikoherbisit olarak bazı patojen fungus türleri saptanmıştır. Hastalıklı tarla sarmaşığı bitkileri 2008, 2009 ve 2010 tarihlerinde, Amasya, Ankara, Çorum, Samsun ve Tokat yörelerinden toplanmıştır. Hastalıklı bitkilerden patojenlerin izolasyonları yapılmış ve morfolojik karakterlerine dayanarak fungusların teşhisleri de yapılmıştır. Tarla sarmaşığı bitkileri iklim odasında 4–5 yapraklı devreye gelene kadar yetiştirilmiş her bir fungus için farklı yoğunluklarda spor süspansiyonları hazırlanmıştır. Küçük spreyler kullanılarak sporlar tarla sarmaşığı bitkilerine, bitkileri tamamen ıslatmak suretiyle püskürtülmüştür. Her bir izolatta hastalık belirtisi oluşma tarihleri kaydedilmiştir. Veriler SAS istatistik programı, kovarians modeli ile analiz edilmiştir. Patojenisite testi sonucunda en fazla hastalık *Stagonospora convolvuli, Colletotrichum linicola* ve *Myrothecium verrucaria* ile inokule edilen bitkilerin yapraklarında saptanmıştır. Bitki boylarında en fazla kısalma *C. linicola* ve bir *Phoma* sp. izolatı ile bulaştırılan bitkilerde olmuştur. Bu sonuçlara göre, *C. linicola'* nın etki potansiyeli olduğu görülmektedir. Bu fungusla ilgili tarla denemeleri tek olarak veya S. *convolvuli* ile birlikte en kısa zamanda yapılmaya başlanmalıdır.

Anahtar kelimeler: Tarla sarmaşığı, Biyoherbisit, Funguslar, Biyolojik mücadele

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