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Stemphylium vesicarium (Wallr.) Simmons as fungal pathogen of false helleborine (*Veratrum album* L.) and it's potential as biocontrol agent[1]

Ünal ASAV[2] **İzzet KADIOĞLU[3]** **Yusuf YANAR**

ÖZ

Veratrum album L. (Adi çöpleme) Üzerinde Bulunan _Stemphylium vesicarium_ (Wallr.) Simmons'un Biyolojik Mücadele Potansiyelinin Belirlenmesi

Bu çalışma *Veratrum album* L. (Adi çöpleme)'un biyolojik mücadelesinde *Stemphylium vesicarium* (Wallr.) Simmons'un etkinliğini belirlemek için yapılmıştır. Trabzon İli mera alanlarında 2009 ve 2010 yıllarında yapılan sürvey çalışmalarında *V. album*'un yapraklarında hastalık belirtileri gözlemlenmiştir. Hastalıklı bitki kısımlarından yapılan izolasyon sonucunda hastalık etmeninin *S. vesicarium* olduğu tespit edilmiştir. *S. vesicarium*'un biyolojik mücadele potansiyelinin belirlenmesi için konukçuya özelleşme testleri ve biyolojik etkinlik çalışmaları yapılmıştır. Biyolojik etkinlik çalışmalarında ise *S. vesicarium*'un 5×10^5 spor/mL konsantrasyonu *V. album*'a 3-4 yapraklı döneminde uygulanmıştır. Bir aylık inkubasyon süresi sonunda *S. vesicarium*'un *V. album* üzerinde %75,25 oranında etkili olduğu tespit edilmiştir.

Anahtar Kelimeler: Biyolojik mücadele, mikoherbisit *Veratrum album, Stemphylium vesicarium*

ABSTRACT

This study was carried out to determine the efficacy of the *Stemphylium vesicarium* (Wallr.) Simmons as a biological control agent on the *Veratrum album* L.(false helleborine). In the survey study which is carried out on grasslands of Trabzon province in 2009-2010, symptoms of a disease were observed on *V. album* leaves. As a result of the isolation from diseased plant parts, *S. vesicarium* was identified. Host selectivity and biological efficiency tests were performed to determine biological control potential of S. *vesicarium* on false hellebore. *V.*

[1] "Trabzon İli Mera Alanlarındaki Önemli Yabancı Ot Türlerinin Yaygınlığı İle Bunların Üzerindeki Fungal Etmenler Ve Etkinliklerinin Saptanması" isimli doktora tezinin bir bölümüdür.

[2] Zirai Mücadele Merkez Araştırma Enstitüsü Müdürlüğü, ANKARA
[3] Gaziosmanpaşa Üniversitesi, Ziraat Fakültesi, Bitki Koruma Bölümü, TOKAT
Sorumlu yazar (Corresponding author) e-mail: unal.asav@tarim.gov.tr
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album seedling with 3-4 leaves stage has been sprayed with *S. vesicarium* 5×10^5 spores/mL. Effectiveness of *S. vesicarium* on *V. album* at the end of one-month incubation period was 75.25%.

Keywords: Biological control, mycoherbicide, *Stemphylium vesicarium*, *Veratrum album*

INTRODUCTION

Veratrum album L. (Melanthiaceae) known as false or white hellebore is perrenial noxious weeds of pasturelands of the world. It can be propagated via seed or rhizoms. The species is distributed over the entire Northern Hemisphere and its origin is Europe. It can commonly found open areas in forest and alpine meadows. *V. album* is an important weed on grazed montane grasslands. These species, which exhibits acute toxicity to mammals, may reach high densities (more than 10 plants/m^2) and displace fodder plants (Spiegelberger et al. 2006). In Turkey, *V. album* is an invasive and poisinous plant distributed especially in pasturelands of Trabzon and the rest of the Black Sea region (Asav et al. 2014).

Mechanical, culturel, physical and chemical weed control methods used in pasturelands are either not effective, costly or too labour intensive. Furthermore, grazing can only be implemented as a management technique when the weed species is non-toxic to the grazing animal. Numerous publications have focused on the chemical and mechanical control of *V. album* (Dorée 1988, Milevoj 1988, Troxler and Rouel 1987). However, broadcast application of herbicides on alpine pastures is not allowed in most European countries, some countries allow treatment of individual plants (e.g. Switzerland, Germany). Recently, efforts have been undertaken to completely banned herbicides from alpine pastures. Therefore, stakeholders are urged to develop new control concepts which are economically affordable and highly selective. In pastures, generally only a single species may cause a problem in a given location (Ammon and Müller-Schärer 1999, Schroeder 1983). In order to protect the many desirable species in the pastures and their biodiversity value, a highly specific control is required. Biological control, namely the use of natural antagonists to reduce weed densities below an economical threshold, may provide an appropriate strategy for management of the most problematic species. So that in recent years environmentaly friendly biological control is being preferred instead of chemical control (Bora 2002, Delen and Tosun 1997, Schaffner et al. 2001, Yiğit 1993).

Numerous pathogens have been isolated from *Veratrum* spp. in different parts of the world. However, no information is available on their effect on *Veratrum* plants. Two rust fungi have been isolated from *V. album*, *Uromyces veratri* (DC.) Schröt. and *Puccinia veratri* Duby. While for both species the teliospores are exclusively associated with *Veratrum* spp., they have a host switch during the aecial stage (*U. veratri*: *Adenostyles*, *Cacalia*, *Homogyne*, *Tussilago*; *P. veratri*: *Epilobium*) (Gäumann 1959). *Puccinia veratri* especially may reach very high infestation levels

under natural conditions. However, due to the cooler conditions at higher altitudes, the epidemic spread of *P. veratri* is slow. It appears that natural infestation by *P. veratri* during the second half of the growing season does not have a measurable impact on resource acquisition by *V. album*, which is probably because the plant has by then replenished the reserves used in shoot production (Schaffner 1994). Besides the above-mentioned fungi, *Mycocentrospora veratri* (Peck) U. Braun (Hyphomycetes) (Morgan-Jones and Phelps, 1995) causes necrotrophic leaf-spots. Indeed, this is the most common and evident pathogen on *V. album* in Switzerland, although its effects on the fitness of *V. album* are unknown.

This study was carried out to evaluate the effectiveness of *S. vesicarium* (Wallr.) Simmons against *V. album*, important weed species of eastern black sea reagon pasturelands and to determine its effects to other plant species which is the basic philosophy of the biological control.

MATERIALS AND METHODS

Isolation and identification of *Stemphylium vesicarium*

A survey was performed at pasturelands of Trabzon in 2009–20010 and infected *V. album* plants were collected and brought to the laboratory in paper bags. The infected plant parts were surface sterilized in 1% sodium hypochlorite (v/v) for 120 s followed by rinsing three times in steril distilled water before being placed directly on to PDA (potato dexrose agar) agar plates. Plates were incubated at 25°C for 4-5 days. At the end of incubation period, mycelial disc 5 mm in diameter was transferred on PDA plate to obtain pure culture. The stock cultures of the isolate were stored at 4 ^{o}C on PDA agar slopes. They were placed in tube culture under oil for long-term storage. Causal organisms were identified by direct examination of conidiospores and conidiospore characteristics (Ellis 1971).

Host specificity tests

Two different methods (whole plant and detached leaf tests) were used to determine the host specificity of the pathogen. Host specificity trails were conducted in Gaziosmanpasa University, Department of Plant Protection Laboratory and growth chamber and the seed germination studies conducted in the greenhouse conditions. List of plant species that were used in host specificity tests was given in Table 1.

Detached leaf tests

Leaves were excised from shoots of the pre-flowering stage of test plants. Three replicates, comprising of three leaves each were tested. These were sprayed evenly with the fungal inoculum using a hand-held, pump spray bottle and then placed in 9 cm petridishes using sterile forceps. Nine replicates of three leaves each were used as control sets to compare the mycoherbicidal activity. The controls received only steril distilled water (SDW) with 0.02% Tween 80. Treatments were carried out within 15 min after detachment from the mother plant. All treatments were kept in a

growth chamber with controlled conditions of 26-28 ºC; 90% relative humidity and 12 h (15000 lx) illumination for a period of ten days. Leaves were rated as healthy or infected.

Table 1. Plant species used in host specificity tests

Family	**Species**	**Local name**
Poaceae	*Agropyron cristatum* (L.) Gaertn.	Crested wheatgrass
Brassicaceae	*Brassica oleracea* L.	Cabbage
Poaceae	*Bromus inermis* Leyss.	Smooth brome
Cucurbitaceae	*Cucurbita moschata* Duch	Zucchini
Asteraceae	*Lactuca sativa* L.	Lettuce
Brassicaceae	*Lepidium sativum* L.	Cress
Poaceae	*Lolium perenne* L.	Englishgrass
Fabaceae	*Lotus corniculatus* L.	Bird's-foot-trefoil
Fabaceae	*Medicago sativa* L.	Clover
Fabaceae	*Phaseolus vulgaris* L.	Bean
Solanaceae	*Solanum melongena* L.	Eggplant
Fabaceae	*Trifolium pratense* L.	Red clover
Fabaceae	*Vicia sativa* L.	Common vetch
Poaceae	*Zea mays* L.	Corn

Whole plant bioassay

Stemphylium vesicarium isolate was grown on Potato Dextrose Agar (PDA) plates at 25±2 ºC for 20-25 days. Spores were harvested by flooding the plates with distilled water and lightly scraping the surface. The resulting spore suspension was filtered through four layers of cheesecloth and adjusted to the appropriate density ($5x10^5$ spores/mL) using a haemocytometer.

Seeds of each plant species were sown in a steam-sterilized, peat moss. Pasture plant seed was sown into the pots to a depth of 10 mm at the rate of 10 seeds per pot while one crop plant seed was sown in each pot at the same depth. When plants reached the 3 to 4 true leaf stage they were sprayed to run-off using a hand-held, pump spray bottle. The seedlings were completely sprayed with a $5x10^5$ spores/mL solution. All treatments were kept in a growth chamber with controlled conditions of 26-28 ºC; 90% relative humidity and 12 h (15000 lx) illumination for a period of four weeks. At the end of incubation period plant with no sypmtops was evaluated as healthy and plant with symptoms was evaluated as infected.

Biological efficacy tests

For biological efficacy test, *V. album* seed was sown in 9×11 cm plastic pots filled with 1:1:1 sand soil and cav manure (v/v). treatments were arranged in a completely randomized design with 4 replications. The controls received only SDW and Tween 80 and Glyphosate-isopropyl-amin (600 mL/da) was used as herbicide control. Test plants were sprayed evenly with the fungal inoculum using a hand-held, pump spray bottle and then placed in a moist chamber as mentioned in whole plant test. Plants

were covered with plastic bags and kept in controlled conditions as described previously. After 24 h, the bags were removed and the plants were placed back in the growth chamber. Plants were rated for disease severity Plant with 5-day interval for one moth on a 11-point scale where;

0= No necrotic spot on leaves,
1= 5% of surface area of leaves was covered with necrotic spots
2= 10% of surface area of leaves was covered with necrotic spots
3= 15% of surface area of leaves was covered with necrotic spots
4= 20% of surface area of leaves was covered with necrotic spots
5= 33% of surface area of leaves was covered with necrotic spots
6= 46% of surface area of leaves was covered with necrotic spots
7= 60% of surface area of leaves was covered with necrotic spots
8= 73% of surface area of leaves was covered with necrotic spots
9= 86% of surface area of leaves was covered with necrotic spots
10= 100% of surface area of leaves was covered with necrotic spots (Falloon et al.1995). In addition, fresh and dry weight of the plants in each treatment were taken. The experiment was conducted 3 times.

RESULTS

Host specificity tests

Host specificity test results were given on Table 2. As it was seen on Table 2, *S. vesicarium* caused disease on *V. album* but not on the other test plants.

Table 2. *Stemphylium vesicarium* host specificity tests

Test Plants	Local Names	Family	Disease symptom	
			Whole plant test	Detached leaf test
Agropyron cristatum	Crested wheatgrass	Poaceae	-	-
Brassica oleracea	Cabbage	Brassicaceae	-	-
Bromus inermis	Smooth brom	Poaceae	-	-
Cucurbita moschata	Zucchini	Cucurbitaceae	-	-
Lactuca sativa	Lettuce	Asteraceae	-	+
Lepidium sativum	Cress	Brassicaceae	-	-
Lolium perenne	Englishgrass	Poaceae	-	-
Lotus corniculatus	Gazal horne	Fabaceae	-	-
Medicago sativa	Clover	Fabaceae	-	-
Phaseolus vulgaris	Bean	Fabaceae	-	-
Solanum melongena	Eggplant	Solanaceae	-	-
Trifolium pratense	Red clover	Fabaceae	-	-
Vicia sativa	Common vetch	Fabaceae	-	-
Zea mays	Corn	Poaceae	-	-
Veratrum album	White hellobore	Liliaceae	+	+

+ indicates presents of the symtom, - indicates no symptom

On the other hand, the pathogen caused necrotic lesion on lettuce leaf besides *V. album* leaf in detached leaf test. Both tests results confirmed that *S. vesicarium* caused leaf spot disease on *V. album*.

Biological efficacy tests

Biological efficacy test results were given on Table 3. As it seen on Table 3, efficacy of *S. vesicarium* and herbicide were 2.5% and 7.5% at the end of five days' incubation period respectively. At the end of 25 day incubation period effects of herbicide and *S. vesicarium* were 100% and 63.25% respectively. At the end of one-month incubation period effect of the *S. vesicarium* on *V. album* reach to 75.25%. It was observed that infected plants grown slower than the control plants. *S. vesicarium* reduced the fresh and dry weight of the plant. The fresh and dry weights of the infected plant were significantly lower than the control plants (Table 4).

Table 3. Biological efficacy of *Stemphylium vesicarium* on *Veratrum album*.

Incubation Period (Day)	**Negative Control (Distilled water)**	**Effect of *Stemphylium vesicarium* (%)**	**Pozitive Control (Glyphosate-isopropyl-amin) (%)**	**LSD Values**
5	0,00b	2,50b	7,50a	4,08
10	0,00c	10,75b	18,75a	2,50
15	0,00c	27,50b	42,75a	12,74
20	0,00c	44,75b	76,25a	19,12
25	0,00c	63,25b	100,00a	12,43
30	0,00c	75,25b	100,00a	14,07

Table 4. Fresh and dry weights of the *Veratrum album* plants tested in biological efficacy tests.

Treaatments	**Fresh weight (g)**	**Dry weight (g)**
Negative control (distilled water)	6,84a*	2,85a
Pozitif Kontrol (Herbicide)	3,12b	0,99b
Stemphylium vesicarium	4,15b	1,38b
LSD ($p \leq 0,05$)	2,63	1,31

* Means followed by the same capital letter within columns indicate there is no significant difference between treatments ($p<0.05$).

There was no significant difference between herbicide treatment and *S. vesicarium* treatments based on the plants' fresh and dry weights.

DISCUSSION

Despite the extensive studies have been conducted on biological weed control as with diseases and pests. The number of commercialized bioherbicides are very limited as compared with commercial herbicides. One of the most important reason of this is forcing the mycoherbicides to compete with chemical herbicides in all aspects which is impossible. Although mycoherbisides have following advantages;

the lack of persistence in water and soil, application for effectiveness against weeds, the possibility of second application depending on the weed output, to be specific host is improved and does not harm the crops, they still can not taken place the herbicide market as it should be.

S. vesicarium was seem a promising biocontrol agent for control of *V. album* with 75.25% efficacy. In both detached leaf and whole plant tests, *S. vesicarium* exhibited host specificity except for lettuce. Previous studies, conducted on pathogenicities of *Uromyces veratri* (DC.) Schröt., *Puccinia veratri* Duby and *Cercosporella veratri* Peck on *V.album* in European and Canada, did not give any data about the efficacy of the pathogens on *V.album*. (Gäumann, 1959; Conners, 1967). Also the study, performed in Georgia for determination of pathogen microorganizms on *V. album*, reported that 25 fungal species including *Ascochyta veratri* Cav., *Cylindrosporium veratrianum* Sacc. & G. Winter, *Fusoma veratri* Allesch, *Marssonina veratri* Ellis & Everh., *Phyllosticta albina* Bub., *Phyllosticta melanoplaca* and *Septoria* sp. caused infection on *V. album* (Gvritishvili et al. 2006).

Although many studies have been conducted on biological control of weed as it seen on plant diseases and pests in recent years, very few micoherbicides were commercialized as it compared with synthetic herbicides. One of the most important reasons this is forcing mycoherbicide to compete with chemical herbicides in all aspects. Mycoherbicides are fungal plant pathogens that are applied as inundative inoculum, as in standard herbicides, to control specific weeds. Even though in some cases, mycoherbicides have proven to be as effective or more effective than chemical herbicides still have not take part in the herbicide market (Daniel et al. 1973). At the end of one-month incubation period, *S. vesicarium* caused 75.25% mortality to *V. album* especially *V. album* is common weed species in pastures in Turkey. So that *S. vesicarium* could be appropriate biocontrol agent of *V. album.* Invitro results of present study looks promising. *V. album* can be controlled by chemical herbicides when a short term solution is required to keep it at acceptable levels or to prevent it invading new areas. For long term control, it is anticipated that biocontrol agents will be integrated into management program.

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