Stachybotrys chartarum as A Bio-Agent to Control *Orobanche* spp

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ABSTRACT

The fungus *Stachybotrys chartarum* was isolated from *Orobanche* seeds, which were collected from different localities of the Nile Delta, Egypt. In pot trials, mycelial suspension, as well as, the fungal filtrate completely prevent the attack of *O.crenata* to faba bean *(Vicia faba)* with. Also, fungal filtrate completely inhibited the germination of *O.crenata* and *O.ramosa* seeds induced by the synthetic germination stimulant GR_{24}. Such effect was attributed to the presence of mycotoxin(s) secreted by the fungus.

INTRODUCTION

Control of broomrape (*Orobanche* spp.) is considered a rather difficult problem because of the tremendous amount of produced minute seeds, their long life span and its requirement of a special stimulant released from roots of particular plants to break seed dormancy.

Biological control by using secondary parasites, fungi or insects, was suggested as a successful control procedure. In this respect, *Alternaria* spp. *Fusarium culmorum, F. gibbsum, F. lateritium, F. moniliform, F. orobanchia, F. oxysporum, F solani., F. sambucinum, F. semitectum, Rhizoctonia solani, Sclerotinia* sp and *Verticillium microsporum* gave good indications as potential biological agents to *Orobanche* spp. (Dufala *et al.*, 1976; Al-Menoufi, 1986; Turhan, 1990; Bedi and Donchev, 1991; Bedi, 1994; Souerborn *et al*., 1994 and Komeil, 2005).

Stachybotrys spp. are saprophytic imperfect fungi isolated from wheat and rice grains and straw, dust, air, and many other biotic and abiotic organic and inorganic materials (Abdel-Hafez and Shoreit, 1985; Khallil, 1990 and Udaiyan, 1992). Some mycotoxins such as zearalenone and zearalenol were isolated and identified as metabolic byproducts of these fungi causing stachybotritoxicosis and other disorders for man and some animals (Servatie *et al.*,1985 and El-Kady *et al*.,1989 and El-Maghraby *et al*., 1991). *S. bisbyi* was isolated from sugarcane causing the red leaf spot disease (Singh *et al*., 1987). On the other hand, *S. atra* is considered as a biocontrol agent for some viral and fungal diseases (Siqueira *et al.*, 1984; Maiss, 1987; Kapoor and kar, 1988 and Ehteshamul-Haque and Ghaffar, 1991).

The present work was designed to investigate the effect of *S. chartarum* (Ehrenb.) Hughes which was isolated from *O. crenata* Forsk seeds, on the germination of *O. crenata* and *O. ramosa L.*, as well as on the ability of *O. crenata* to parasitize faba bean (*Vicia faba*) plants.

MATERIALS AND METHODS

A fungus associated with ungerminated *O. crenata* seeds (in the synthetic germination stimulant GR_{24} medium) was isolated, purified on potato dextrose agar (PDA) medium and identified by Centraalbureau Voor Schimmelcultures, Braan, the Netherlands.

Fungal mycelial mats and filtrates were prepared by inoculating 250 ml conical flasks containing 100 ml of potato dextrose liquid medium with disks (0.5 cm diameter) from 21-days old fungal cultures growing on PDA at 20^{o}C. Flasks were incubated for 21-25 days at 20^{o}C. Mycelial suspension was prepared by blending the harvested mycelial mats in distilled water (1 mat/100 ml) to be used in pot experiments. Fungal filtrate was sterilized by Zeitz filter to be used in the germination and pot experiments. The effect of filtrates heated at 60^{o}C for 30 mins. or autoclaved (121^{o}C for 15 mins.) on the germination of *O .crenata* and *O. ramose* seeds, as well as, their effect on the number of *O. crenata* plants attached .to faba bean roots were also investigated

To study the effect of fungal filtrate on seed germination of *O. ramosa* and *O. crenata*, preconditioned seeds were placed onto filter papers in Petri dishes containing 3 ml of the synthetic germination stimulant GR_{24} alone or mixed with fungal filtrate (1:1, v:v). Petri dishes were then incubated for 4-5 days at 20^{o}C before counting the germinated seeds. Treatments were replicated six times in a complete randomized block design.

Pots (25-cm diameter) with 30 days old faba bean plants (4 plants/pot) were treated with the mycelial suspension or filtrates (100 ml/pot). Pots were previously infested with *O. crenata* seeds (0.025 g/pot = 2500 seeds). Pots uninfested with *O. crenata* seeds, were also treated with mycelial suspension or filtrate. Untreated pots, infested or uninfested with *O. crenata* seeds, were served as control treatment. Treatments were replicated ten times in a complete randomized block design. Number of *Orobanche* attachments on faba bean plants was counted 90 days after sowing.

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RESULTS AND DISCUSSION

Data (Table1) revealed that *O. ramosa* and *O. crenata* seeds are readily germinated with 1 PPM GR_{24} (83.4 % and 71.0 %, respectively). By adding the *S. chartarum* filtrate to the GR_{24} 2 PPM solution (1:1,v:v), the germination of *Orobanche* seeds was completely inhibited (0.0 %). Slight germination was detected when fungal filtrate was previously heated (5.3 % and 2.8 % for *O. ramosa* and *O. crenata*, respectively) or autoclaved (6.7 % and 3.1 % for *O. ramosa* and *O. crenata*, respectively). Statistical analyses revealed that reduction in germination due to the fungal filtrates is highly significant.

In pots treated with either mycelial suspension of *S. chartarum* or its filtrate, the growing faba bean plants were completely free from broomrape attachments. Faba bean plants in untreated pots were parasitized by 4-9 broomrape plants with an average of 5.7 parasite/host plant. Either the mycelial suspension or the fungal filtrate did not affect the growth of faba bean plants.

The obtained results revealed that the *S. chartarum* secrets a thermostable mycotoxin(s), which might inhibit the germination of *Orobanche*, seeds or block the stimulatory effect of GR_{24}. It is known that *Stachybotrys* spp produce certain mycotoxins such as zearalenone and zearalenole(El-Kady *et al*.,1989). The inhibitory effect of *S. chartarum* on *Orobanche* seed germination might be due to these mycotoxins.

According to the above mentioned results it concluded that the fungus *S. chartarum* might be a promising biocontrol agent for *Orobanche*. Further investigations are needed to verify such results and to avoid any harmful effect(s) on other living organisms.

Table 1. Effect of *Stachybotrys chartarum* filtrate (FF) on the germination of *Orobanche* seeds treated with the synthetic germination stimulant GR_{24}

Treatment	Germination Percentage	
	O. ramosa	***O. crenata***
GR_{24} 1 ppm	83.4	71.0
FF	0.0	0.0
FF + GR_{24}[1]	0.0**	0.0**
Heated FF + GR_{24}[1]	5.3**	2.8**
Autoclaved FF + GR_{24}[1]	3.1**	6.7**
L.S.D. 0.01.	9.3	8.5

[1]FF + GR_{24} (2 ppm) at the rate of 1:1 (V:V)

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الملخص العربي

إستاكيبوتريس كارتارم مبيد حيوى للهالوك

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تم عزل الفطر إستاكيبوتريس كارتارم من بذور هالوك سبق تجميعها من عدة مناطق من دلتا النيل فى جمهورية مصر العربية. إستخدمت إفرازات الفطر النامى على بيئة سائلة لدراسة تأثيرها معمليا على إنبات بذور الهالوك أوروبانكى كرينا تا وأوروبانكى راموزا ى فى وجود مادة GR_{24} المخلقة لتنبيه إنبات بذور الهالوك.

بينت النتائج أن إفرازات الفطر الطبيعية والمخففة بالماء (1:1) تمنع تمام إنبات بذور الهالوك بنوعيه.

كذلك إستخدمت إفرازات الفطر و المعلق الميسليومى له فى دراسة للتعرف على تأثيره على قدرة الهالوك أوروبانكى كرينا تا على إصابة نباتات الهالوك النامية فى أصص تحتوى تربة معدية ببذور الطفيل. وقد بينت النتائج أن معاملة التربة بإفرازات الفطر ومعلقه الميسيليومى منعت تماما إصابة الفول بالهالوك.