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Potential Biological Control of *Orobanche* by Fungi Isolated from Diseased Specimens in Jordan

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Species of the genus *Orobanche* are parasitic flowering plants, holoparasites, which cling to the roots of green plants. Their tiny seeds (200 × 250 μm) germinate in response to chemical stimuli produced by host and some non-host plants. Successful contact with their host leads to development of haustoria for obtaining water and food. The shoots above the ground expose flowers and disseminate seeds. Several samples of *Orobanche ramosa, O. crenata, O. cernua,* and *O. egyptiaca* were collected from different localities in Jordan. These samples showed one of the following disease symptoms: dry rot at the base of the stem; general deterioration and expanded lesion from base upward; soft tissue maceration of stem; and black rot of flower parts with incomplete maturation of the ovary and seeds. Isolation from diseased stems and seeds was made on three different mycological media. Several fungi were isolated, mainly, *Fusarium* spp., *Alternaria alternata, Rhizoctonia* sp., *Dendrophora* sp., *Chaetomium* sp., and an ascomycetus fungus with a perithecium. Pathogenicity tests showed that *Fusarium* spp. and *Alternaria alternata* attacked healthy living tissue of *Orobanche* spikes. These fungi caused lesions of black soft rot and complete deterioration within 5-7 days. They also attacked *Orobanche* seeds, arresting their germination and causing maceration of non-germinated and germinated seeds after 5-7 days of incubation. Meanwhile, *Dendrophora* sp. and *Chaetomium* sp. caused limited lesion at first, but were able to colonize the tissue as it aged and senesced. This study showed the presence of a potential endogenous pathogenic fungi in Jordan, which can be investigated as a biological control for *Orobanche*.

Keywords **:** *Alternaria alternata*, biological control, *Fusarium* sp., *Orobanche* spp.

Species of the genus *Orobanche* are parasitic flowering plants, holoparasites, which cling to the root of their host green plants. Their tiny seeds (200 × 250 μm) germinate in response to stimuli produced by the host plants. They develop haustoria on the root of their hosts. The shoots above ground expose flowers and facilitate seed dissemination. These parasites represent an increasing threat to several vegetable and fruit crops (Al-Khazraji et al., 1987; 1989) in a wide range of cultivated lands, leading to yield losses of up to 100% in the host plant in several places worldwide (Sauerborn, 1991), particularly in Jordan. These parasites are also widely distributed in other Arab countries, as well as in countries within the arid and semi-arid regions (Parker, 1994). Combating and controlling these parasites have been a difficult task due to the narrow margin of selectivity of available herbicides between the host and the parasite in case of chemical control (Garcia Torres et al., 1994). Control is also difficult due to the nature of this parasite, as it produces a vast number of seeds (Saghir et al., 1973a). They are capable of staying dormant in the soil for many years waiting for the germination stimulant being exuded from the plant root (Saghir et al., 1973b). The germinated seed has to be in contact with the root to develop a haustorium or tubercule (Hameed and Foy, 1991), and shoot(s). This process takes about 5-7 weeks (Saghir et al., 1973b), which means that the host plant has been suffering for the duration of the period prior to the emergence of the parasite. The negative impact of the parasite upon the host plant is closely related with the developmental stage of both parasite and its host (Manschadi et al., 1996). Therefore, considerable efforts are needed in adopting control measures against non-germinated and/or germinated *Orobanche* seeds, in order to prevent initiation of infection such as deep plowing (Petzoldt et al., 1994). Further, the use of trap crop or decoy plants (Saghir et al., 1973b), and the utilization of inherited resistance genetic resources against the infection by these parasites are needed for control. Biological control of *Orobanche* and other weeds (Fayadh et al., 1990) has been advocated for integrated management, which includes measures such as the use of insect predators on *Orobanche* (Kruschel and Klien, 1995) and pathogenic fungi (Thomas et al., 1998).

The search for biological control agents against Orobanche (Bedi, 1991) and *Striga* spp. (Kroschel et al., 1996) was prompted by field observation of the disease on parasitic seed plants and the need to isolate the causal pathogen(s).

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The potential of these agents was further investigated for their feasibility in future biological control programs (Thomas et al., 1998). Many commercial biological control agents based on the use of pathogenic fungi have been developed for weed control in, among others, rice and soybean (Bowers, 1986). In Jordan, field observations revealed a wide incidence of diseased *Orobanche* plants (personal observation). These cases involved several *Orobanche* species on different commercial crops. Therefore, this study aimed to isolate pathogenic fungi and investigate their pathogenicity potential against the vegetation and seeds of *Orobanche* in Jordan.

Materials and Methods

Collection of diseased *Orobanche* specimens. Stems of *Orobanche ramosa, O. crenata, O. cernua,* and *O. egyptiaca* were collected during several field trips in the northern region of Jordan. These stems were sampled for diseased *Orobanche* plants showing symptoms such as wilting, dry or soft rot at the base of the stem, and complete blight of the stems with black floral parts and ovules. These samples were collected from tomato, eggplant, and faba-bean vegetable crops infected with *Orobanche.*

Isolation of the disease-inciting fungi. Segments (1-2 cm long) of diseased stem and seeds were surface-sterilized by placing them inside 10% sodium hypochlorate solution for 2-3 min, and then rinsed with sterilized distilled water. Surface-sterilized materials were plated on acidified potato-dextrose agar (PDA), malt agar (MA), and corn meal agar (CMA) mycological media. Fungal growth associated with diseased *Orobanche* was isolated into pure culture, and characterized and identified to genus and species.

Pathogenicity test of the isolated fungi. The isolated fungi were inoculated on healthy stem segments and seeds in order to test the pathogenicity of the isolated fungi on *Orobanche* materials. Five healthy stem segments of 2-3 cm long and seeds were first surface-sterilized with hypochlorate solution prior to inoculation. The seeds were preconditioned on moist filter paper inside Petri dishes for at least 8-10 days at 25°C of incubation. This treatment prepared them to respond to the germination stimulant, GR24 (2 ppm solution, first dissolved in 1 ml acetone and then made up to volume in water) with which they were irrigated later on. Stem segments were placed on moistened filter paper inside plastic Petri dishes, and the seeds were spread on a seed lawn on the surface of the water agar in separate plates. About 250 seeds per plate and 3-4 plates were used for each kind of seed. Each stem segment was inoculated by transferring a tiny portion of the fungal growth with the aid of an inoculating needle to the surface of the agar and making 3-5 pricks on that spot, then placed in a moist chamber and incubated inside an incubator at 25°C. These segments were observed for disease development within the next 2 days and again 5 days later.

Disease assessments. Disease incidence and severity were rated on the basis of the symptom development and an arbitrary scale ranging from zero (0) meaning no disease observed, to 100 where the whole segment was affected by the fungus. The range of disease severity was recorded according to the scale on each segment of the *Orobanche* species and for each fungus. The seed lawns were irrigated with 2 ml of the GR24 solution. Twelve hours later, the plates were inoculated by transferring 3 mm discs from the PDA culture plates of these fungi into the center of the seed lawn on water agar plates. Crude culture filtrates from liquid culture of the fungi were also tested for the activity against *Orobanche* seed. In this case, the filtrates were either used to fill holes (5 mm in diameter) made at the center of the water agar seed lawn, or 2 ml of the culture filtrates were poured on the surface of that seed lawn. The effects of fungi upon the seeds were determined by counting percent germination, in the whole plate and in the three zones, 1 cm apart around the central hole for the culture filtrate treatment. Health condition and physical consistency of non-germinated as well as germinated seeds and their radicles were recorded.

Mass inoculum of the pathogen and its effect on soil. In order to get large masses of fungal growth, they were grown on wheat grains soaked in water for 12 h, and then sterilized by autoclaving. Wide mouth glass jars (1-kg honey jars) with metal caps were used as chambers. The caps were punched at the center to make a

Table 1. Incidence of fungi associated with *Orobanche* stems and seeds from open fields in Jordan showing disease symptoms

Isolated fungi	Fungi isolated from diseased *Orobanche* materials[a]							
	O. ramosa		*O. cernua*		*O. egyptiaca*		*O. crenata*	
	Stem	Seed	Stem	Seed	Stem	Seed	Stem	Seed
Alternaria alternata	+	+	−	−	+	+	+	+
Fusarium sp.	+	+	+	+	+	+	+	+
Dendrophoma sp.	−	−	+	−	+	−	−	−
Rhizoctonia sp.	−	−	+	−	−	−	+	−
Cheatomium sp.	−	−	−	−	−	−	+	−
Pullularia sp.	−	−	−	−	+	+	−	−
Aspergillus sp.	−	−	−	−	+	+	−	−
Penicillium sp.	−	−	+	+	−	−	+	+

[a] +: isolated, −: not isolated.

2-cm hole which was plugged with cotton. The jars were one-third filled with soaked wheat grains and then sterilized by autoclaving and used for culturing the fungi. Fungal growth on autoclaved wheat grain was mixed in soil and used for planting tomato seedlings inoculated with *Orobanche* seeds in order to test the pathogenicity of the fungi against the parasite and the host plant.

Fig. 1. Macroconidia of *Fusarium* sp. isolated from diseased *Orobanche* and used as biological control agent against the parasitic weed.

Results and Discussion

Isolated fungi from diseased *Orobanche* specimens include: *Fusarium* sp., *Alternaria alternata, Rhizoctonia* sp., *Dendrophora* sp., *Chaetomium* sp., and an ascomycetus fungus with perithecium (Table 1). *Fusarium* sp. (Fig. 1) was the common isolate from all samples investigated. *A. alternata* was isolated from *O. ramosa, O. eygyptiaca,* and *O. crenata.* However, it was not found from specimens of *O. cernua.* Other fungi associated with the disease were observed but were considered to be secondary invaders.

The first two fungi, *A. alternata and Fusarium* sp., attacked living tissue of *Orobanche* stem segments, causing

Table 2. Pathogenicity of the isolated fungi on healthy stem segments of *Orobanche crenata*

Isolated fungi	Disease ratings of fungal infection on *O. crenata* after 2 and 5 days of incubation									
	Fungal growth (%)[a]		Tissue maceration[b]		Tissue discoloration[c]		Disease incidence (%)		Disease severity[d]	
	2d	5d	2d	5d	2d	5d	2d	5d	2d	5d
Alternaria alternata	+	100	–	+	*	**	+	+	10	100
Fusarium sp.	+	100	–	+	*	**	+	+	10	100
Rhizoctonia sp.	+	95	–	+	*	**	+	+	10	95
Dendrophoma sp.	+, –	94	–	+, –	*, –	*	+, –	+, –	0	80
Control	–	–	–	–	–	–	–	–	0	0

[a] +: growth; –: no growth.
[b] +: tissue macerated, –: no tissue maceration.
[c] Color change of tissue to normal wax color (*) or black (**); –: no color change.
[d] 0=no disease to 100=complete colonization.

Table 3. Pathogenicity of isolated fungi on healthy stem segments and seeds of *Orobanche ramosa*

Isolated fungi	Disease ratings of fungal infection on *O. ramosa*				
	Stems			Seeds	
	Fungal growth (%)	Disease incidence (%)[a]	Disease severity (%)[b]	Seed germination (%)	Loss in seed texture[c]
Alternaria alternata	100	+	100	0	100
Fusarium sp.	100	+	100	0	100
Dendrophoma sp.	80	+	90	0	100
Rhizoctonia sp.	80	+	90	0	100
Cheatomium sp.	50	+	50	25	50
Pullularia sp.	0	–	0	78	0
Aspergillus sp.	0	–	0	78	0
Penicillium sp.	0	–	0	78	0
Control	0	–	0	78	0

[a] + = 100%; – = 0.
[b] 0 = no disease to 100% complete colonization.
[c] Loss in integrity of mounted seed (0 = no loss to 100 = complete loss) when pressed on microscopic slide.

black lesion, soft rot, and complete deterioration within 5-7 days (Tables 2 and 3). Meanwhile, *Dendrophoma* sp. and *Rhizoctonia* sp. caused 80-90% disease severity, while *Aspergillus* sp. and *Penicillium* sp. caused limited lesion.

Both *Fusarium* sp. and *A. alternata* were capable of achieving complete colonization of the *Orobanche* segments and resulted in 100% disease severity. On the other hand, their effect on *Orobanche* seed germination was also significant, as there was no seed germination. This may indicate the potential of these fungi to cut down on the inoculum of this parasite (Table 3). They have attacked the seed and resulted in complete loss in their texture. Results of the *Chaetomium* sp. treatment may be due to the fact that this fungus attacked the deteriorating tissue and seeds only.

Table 4 shows that the crude culture filtrate may contain some inhibitory factor(s) against the *Orobanche* seed germination. Germination was reduced by about 80% compared to the control. The crude culture filtrate was run through a Millipore filter and applied under clean conditions. This kind of effect of the culture filtrate was further substantiated by the results of the central hole technique (Table 4). The *Orobanche* seed germination in zone A (1 cm apart from the center) was drastically reduced from about 52% to only 10%. In this zone, the seeds are exposed to the diffusing materials of the culture filtrate first and in its most concentrated form. The seeds in zone B (2 cm apart from the center), however, are exposed later than the ones in zone A, and the diffusing materials got more diluted. At zone C (3 cm apart from the center), seed germination is similar to that in the control treatment, which shows that the

Fig. 2. Orobanche seed infected with *Fusarium* sp. showing extensive damage on the seed.

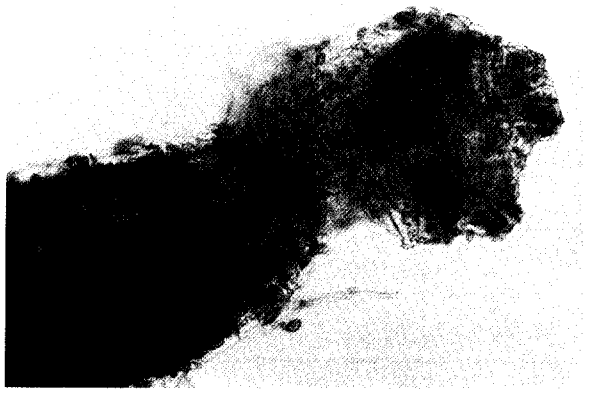

Fig. 3. Germinated *Orobanche* seed which is heavily invaded by the fungus *Alternaria alternata*. This fungus was used as a biological control agent against this parasitic weed.

Fig. 4. Conidia of *Alternaria alternata* formed by this fungus on infected *Orobanche* seed.

Table 4. Effects of crude culture filtrates on *Fusarium* sp. and *alternaria alternata* on *Orobanche* seed germination

Culture filtrate	% Seed germination					
	Applied in the central hole[a]				Poured on seed laum[b]	
	Treated (apart from the central hole)				Control	Treated
	Control	1 cm	2 cm	3 cm		
Fusarium sp.		–[c]	–	–	36.0	3.7
Alternaria alternata	56.8	9.8	24.0	51.7	57.0	7.7

[a] A culture filtrated was applied in a central hole (5 mm in chiamete) with 2 ml of the GR24 solution (2 ppm of the fungal culture filtrate).
[b] Two ml of the culture filtrate solution (2 ppm) was pound throughout water agar on which seeds were layered.
[c] –: data missing.

Table 5. *Orobanche* infection on the root of tomato in a pot experiment as indicated by number of shoots emerged above the soil surface and tubercules (infetion attachment)

Crop/Treatment[a]	Number of *Orobanche* shoots and tubercules			
	O. ramosa		*O. crenata*	
	Shoots	Tubercules	Shoots	Tubercules
Control	15	43	1	6
Fusarium sp.	0	3	0	0
Alternaria alternata	0	0	–[b]	–
Rhizoctonia sp	0	0	–	–

[a] Fungal inoculm was incorporated in soil at time of transplanting.
[b] data not arailable

effect may be due to some factor(s) that were diffusing from the central hole where the culture filtrate was placed.

Treatment of healthy *Orobanche* tissues and seeds with the fungi resulted in disease conditions on *Orobanche*. Also, their culture filtrates resulted in the reduction in germination and caused maceration of non-germinated and germinated *Orobanche* seeds after 5-7 days of incubation.

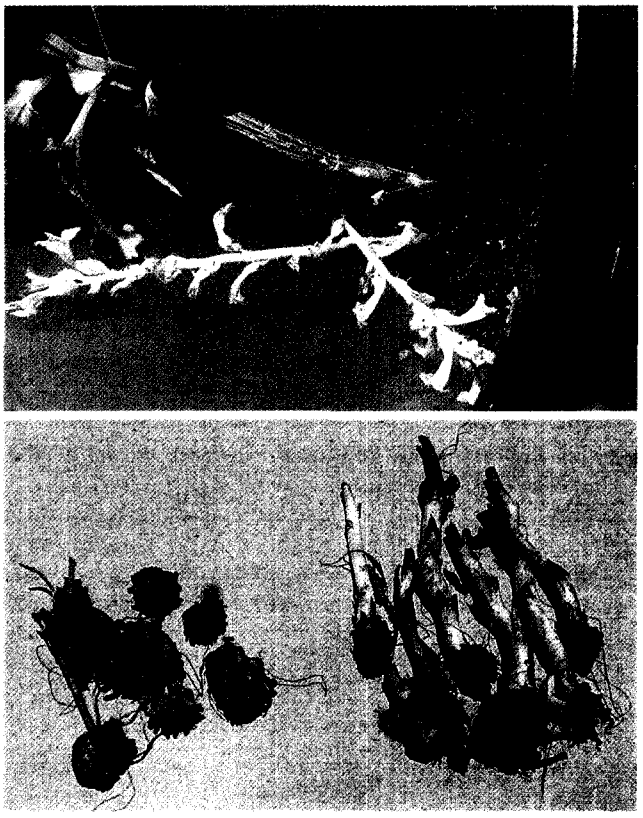

Fig. 5. Orobanche shoots (above) next to their host plant. The shoot inoculated with *Alternatria alternata* shows complete death, necrosis, of stem tissue and damping off of the shoot. Under ground *Orobanche* (below left) from *A. alternata* treatment compared with healthy non-inoculated control (below right).

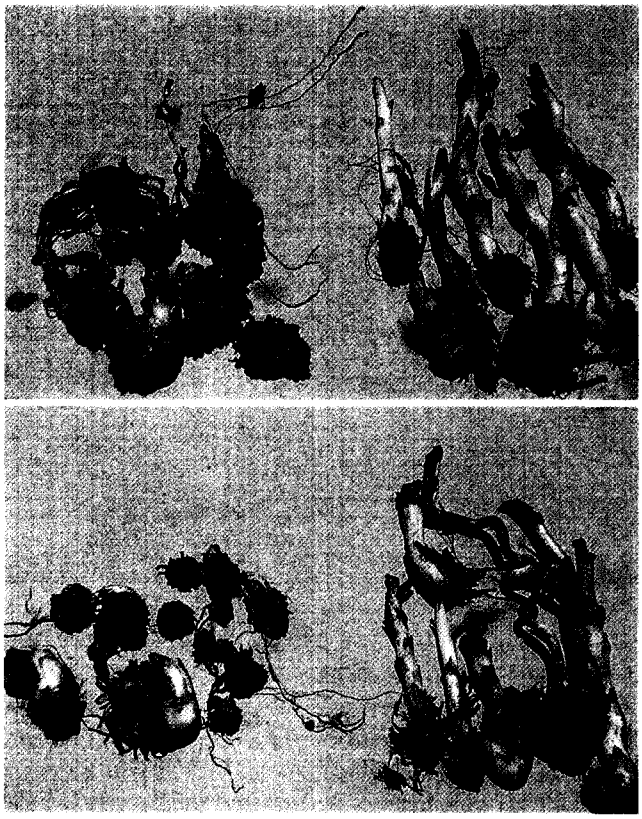

Fig. 6. Orobanche tubercules and sprouts from potted soil infested with *Rhizoctonia* sp. (above left) and *Fusarium* sp. (below left) compared with healthy orobanche of the non-treated control.

These kinds of results are useful for future biological control investigations.

The introduction of the fungal growth as mycelium on wheat grain in the potting soil of tomato transplants showed that *Orobanche* shoots have emerged in the control pots only after 5-7 weeks from transplanting (2-3 *Orobanche* shoots per plant) (Table 5). However, there were no pathogenic effects against tomato by the fungi tested. Carefully washed root systems showed that the number of attachments of *Orobanche* occurred only on the roots of the control.

Results of the greenhouse experiment showed that all fungi isolated from diseased *Orobanche* used as biological control agents were not pathogenic on the host plant tomato, yet they showed different degrees of pathogenicity against the parasite (Table 6). The isolate of *A. alternata* showed the best results in cutting down the number of tubercules of *Orobanche* and eventually the disease incidence of tomato under greenhouse conditions. However, the isolate of the *Fusarium* sp. was also effective in controlling this parasite. The other species were not as effective against this parasite. These results indicate the potential of biological control of *Orobanche* by fungi isolated in Jordan.

Table 6. Efficacy of biological control agents, *Fusarium* sp., *Alternaria alternata*, and *Rhizoctonia* sp., under greenhouse conditions for *Orobanche* on tomato plants

Treatments	Host and parasite growth						
	Tomato				*Orobanche ramosa*		
	Shoot			Root	Shoots	Tubercules	
	Height (cm)	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Number	Fresh weight (g)	
Control	31	35	8	35	0	0	41
Control + *Orobanche*	16	24	4	8	104	56	13
Fusarim sp.	54	120	16	0	0	0	84
Fusarium sp. + *Orobanche*	41	98	15	0	22	40	59
A. alternata	49	120	21	0	0	0	66
A. alternata + *Orobanche*	40	82	20	0.2	8	50	41
Rhizoctonia sp.	38	100	18	0	0	0	53
Rhizoctonia sp. + *Orobanche*	34	87	15	0.2	23	45	57

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