IJABBR- 2014- eISSN: 2322-4827

International Journal of Advanced Biological and Biomedical Research

Journal homepage: www.ijabbr.com

Original Article

Biological Control of *Orobanche Ramosa* by *Fusarium Solani*

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ARTICLE INFO

Article history:
Received: 06 August, 2014
Revised: 27 September, 2014
Accepted:19 October, 2014
ePublished: 30 November, 2014

Key words:
Biocontrol
Broomrape
Tomato
Melon
Watermelon

ABSTRACT

Objective: The main objective of this study was to screen and evaluate the potential of fungi that isolated from *Orobanche ramosa* with potential as biological control agents against the parasitic weed, in laboratory and greenhouse experiments. **Methods:** In this investigation, isolates of *Fusarium solani* were recovered from diseased broomrape collected from tomato, eggplant, melon, and watermelon fields in Southern Khorasan. The pathogenicity of isolates was evaluated on *O. ramosa* stem and seed. *Fusarium* damaged all of the developmental stages and prevented the damage that broomrape causes to plants. **Results:** The germination of inoculated seed was significantly reduced, germ tubes of microconidia penetrated all parts of the thick, complex seed testa, and seed contents were destroyed. Pathogenicity tests indicated that *F. solani* caused lesions of black soft rot and complete deterioration within 5-7 days (100%). They also attacked *Orobanche* seeds, arresting their germination and causing maceration of non-germinated and germinated seeds after 5-7 days of incubation. This is the first investigation of the effects of *F. solani* on host plant interactions with broomrape in melon.

1.INTRODUCTION

Orobanche ramosa (broomrape) is a widespread parasitic weed of many Solanaceouse crop species, such as tobacco or tomato, and attaches to many other species, including ornamentals and weeds. It is mainly distributed in the Mediterranean area, central Europe, northern Africa, and the Middle East (Press and Graves, 1995).Many fungi were isolated from diseased Orobanche plants, and some of them proved to be mycoherbicides for biological control of broomrapes (Galdames and Diaz, 2010). *F. oxysporum* f.sp. *orthoceras* obtained from diseased *O. cumana* tested in soil with sunflower as a host plant was able to reduce the number of attached and emerged *Orobanche* seedlings by about 90% (Bedi and Donchev, 1991). *F. oxysporum* f. sp. *orthoceras* on *O. cumana* was also tested by Thomas et al. (1998). *F. arthroporioides* and *F. oxysporum* isolated in Israel from *O. aegyptiaca* were shown to be effective in reducing broomrape growth (Thomas et al., 1999). Muller -Stover (2001) showed two isolates of *Ulocladium botrytis* and *F. oxysporum* f.sp. *orthoceras* caused necroses on both *O. cumana* and *O. crenata* (Müller-Stöver et al., 2002).The development of an appropriate formulation which allows successful application of fungal propagules will determine the success of *Fusarium* in agriculture applications. The encapsulation of fungal propagules in a solid matrix 'Pesta' showed 70% reduction of Orobanche emergence when wheat flour kaolin granules containing chlamydospore rich biomass was applied. Some toxins produced by fungi of the genus *Fusarium* proved to be able to inhibit germination of *O. ramosa* seeds, and their practical use in integrated strategies of parasitic plant management has been proposed (Zonno

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and Vurro, 2002). Extracts from the liquid culture of fifty-three isolates of fusarium species having the ability to inhibit the induced germination of *O. ramosa* seeds (Abouzeid et al., 2004). The effects of *Fusarium* on host plant interactions with broomrape in melon have not previously been studied. Considering the importance of *O. ramous* in the east of Iran and the lack of research on fungi associated to *Orobanche* spp., the main objective of this study was to screen and evaluate the potential of fungi isolated from *Orobanche* with potential as biological control agents against the parasitic weed, in laboratory and green house experiments.

2. MATERIALS AND METHODS

To isolate pathogens of *Orobanche spp*, stems with such symptoms as wilting, dry, or soft rot at the base of the stem, and complete blight of the stems with black floral parts and ovules were collected in the eastern region of Iran during 2010-2011 growing season. These samples were obtained from several fields of tomato, eggplant, melon, and watermelon. Segments (1-2 cm long) of diseased stems were surface-sterilized by immersion in 10% sodium hypochlorite solution for 2-3 min, and then rinsed with sterilized distilled water and plated on acidified potato-dextrose agar (PDA), and cornmeal agar (CMA). Colonies, which grew from the stem pieces of *Orobanche,* were purified and identified to species. Isolates were tested for pathogenecity by inoculating seed or five 2-3 cm long apparently healthy stem segments Orobanche. Stem segments were placed on moistened filter paper inside plastic Petri dishes. Each stem segment was inoculated by transferring a tiny portion of the mycelia 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 (Andolfi et al., 2005; Sharma et al., 2010). Fifteen milligrams of seeds were sprinkled on a glass microfiber filter paper disc (Whatman, 9 cm diameter) in a Petri dish. The seeds were incubated in the dark for 7 d, after which at least 100 seeds, were excised and transferred to a second Petri dish .To induce seed germination, 3 ml of tomato root-exudates were added to seed on filter paper. The exudates were collected by transferring 14 d-old seedlings of tomato to Erlenmeyer flasks filled with tap water (250 ml). The flasks were covered with black polyethylene to exclude light from the root zone. After 3 d, the liquid enriched with tomato root exudates was harvested by filtration through glass microfiber filter paper and stored at 20 °C until required. The seeds were incubated in the same environmental conditions for 7 d, when a further percentage germination was determined by evaluating 400 seeds under a binocular (Thomas et al., 1999). Disease incidence and severity were rated based on the symptom development and a disease rating scale ranging from 0 (no disease observed) to 100 (entire stem segment of orobanche were rotted). The seed were irrigated with 2 ml of the GR24 (synthetic germination stimulant) solution. Twelve hours later, the plates were inoculated by transferring 3 mm discs from the PDA culture plates of these fungi into the centre 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 centre 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. 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 and melon seedlings inoculated with *Orobanche* seeds in order to test the pathogenicity of the fungi against the parasite and the host plant(Sharma et al., 2010; Andolfi et al., 2005).

3. RESULTS

Forty-six isolates of fungi were recovered from infected *Orobanche*. Forty colonies were woolly to cottony with cream to white aerial mycelium and a cream reverse. Sporodochia were blue-green or blue, conidiophores had simple or branched monophialides, macroconidia were moderately curved, thick-walled, usually 3–5 septate, microconidia were one to three-celled, chlamydoconidia occurred both singly and in pairs. This isolates distinguished as *F. solani* (Leslie et al., 2006). *F. solani* were recovered from infected plants, ten isolates from melon and ten from watermelon, ten isolates from tomato, and ten isolates from eggplant fields. Five isolates of *Alternaria alternate* were recovered from infected tissues. In one sample of *Orobanche* from watermelon, one *Pythium* isolate was recovered. Pretest of *F. solani* pathogenecity on stem of *O. ramosae* showed all isolates were able to rot o. ramosae stems. Two isolates from each host were selected for other pathogenecity tests. *F. solani* attacked living tissue of broomrape stem segments, causing black lesion, soft rot, and complete deterioration within 5-7 days. *Fusarium* isolates were capable of achieving complete colonization of the broomrape segments and resulted in 100% disease severity. Forty percentage of *Orobanche* seeds were germinated after GR24 treatment. The moderate germination rate in the control treatment can be explained by seed dormancy. *F. solani* reduced germination of broomrape seeds from 40% in the control to 5% in the inoculated treatment. Inoculated seeds of broomrape failed to germinate due to colonization by *F. solani*. The fungus was very effective at colonizing seeds

of *Orobanche in* v*itro*. One day after inoculation and incubation at 6 °C, the seeds were overgrown by mycelium. The introduction of the fungal growth as mycelium on wheat grain in the potting soil of tomato and melon transplant showed that *Orobanche* shoots have emerged in the control pots only after 5-7 weeks from transplanting (2-3 *Orobanche* shoots per plant). *Alternaria* and *Pythium* isolates were non-pathogen on seed and *Orobanche* segments. However, no pathogenic effects were observed for the fungi against tomato. 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 melon and tomato, yet they showed different degrees of pathogenicity against the parasite.

4. DISCUSSION

This is the first study on biocontrol fungi from *Orobanche* spp. in watermelon and melon fields in Iran but broomrape biocontrols of second groups, tomato and eggplant, were studied in northern and centre of Iran before that. Our data revealed that *F. solani* was the major mycoherbicide on broomrape in the eastern of Iran. Seed germination of *Orobanche* spp. is induced by chemical stimulants that are part of the host root exudates. The germinating seeds can be inhibited by *F. solani*. It is likely that the combined attack on seeds, germ tubes, and tubercles in soil by *F. solani* was responsible for the reduced number of broomrape shoots in pre-plant inoculated pot trials(Bedi and Donchev, 1991). Shoots became infected underground, leading finally to the reduced rate of *Orobanche* emergence and an increase in tomato and melon dry matter. At present, the high level of virulence of *F. solani* on the underground stages of the parasite and the inability of herbicides to control broomrape on tomato and melon makes further development of the fungus into a biocontrol agent very promising and vital for the control of this serious weed pest. The results indicated that seeds could be infected in the field after application of the fungus suggests that it may not be necessary to apply the inoculums during crop growth. This could lower the Orobanche seed bank every season, giving a special advantage to the mycoherbicide approach. Seeds of O. ramosa buried in a field naturally infested with a pathogenic Rhizoctonia, showed reduced germination after two months (Duafala et al., 1976). Inundative soil incorporation of fungi combined with crop lines resistant to the holoparasitic weed might be a useful strategy of integrated management of broomrape with potential for long-term reduction of its seed bank. In southern of Iran ,pest control experiments have shown that *F. oxysporum* reduces the amount of tobacco plant parasites by over 70% and achieves yield increases of approximately 80% (Mazaheri and Vaziri, 1991). Mazaheri and Ershad tested *F. solani* for controlling broomrape in tomato and cucumber fields(Mazaheri and Ershad, 1995) .Mehrabi et al.(2006) isolated *F. solani , R. solani, Alternaria spp., Ulocladium atrum, F. oxysporum, F. semitectum, Verticillium allbo-atrum, F. equiseti, Bipolaris austransis* from *O. aegyptiaca* from centre of Iran(Mehrabi Kushki, 2006). Haj seyed hadi and his co-worker showed pathogenicity of *F. solani* on *O. aegyptiaca*(Hadj Seyed Hadi et al., 2005.) . The effects of *F. oxysporum* from Karaj on broomrape seed germination showed the synthetic compounds such as GR60 for stimulating *O. cernua* seed germination in absence of host plant and biocontrol agents such as some isolate of *F. oxysporum*, can be used for reduction of the broomrape seed bank and control of this parasitic weed(Hasannejad et al., 2006). The highest inhibition of seed germination obtained in 10(5) spores/ml. With increasing of suspension concentrations, inhibition percent was reduced and mortality of seeds germ tube was increased(Hasannejad et al., 2006). A trial was conducted to determine the most effective nutritional regimes for enhancing conidiation, disease incitement and desiccation tolerance of *F. oxysporum* isolates(Ghotbi et al., 2011). The highest rate of *P.aegyptiaca* control (100% mortality) was achieved by spores of isolate 507 cultured in RS containing 5% (v/v) of glycerol and SD without glycerol. The use of RS with glycerol is more economic and effective for mass production than using SD medium(Ghotbi et al., 2011). Researcher isolated *F. solani, F. oxysporum, F. moniliforme , Sclerotinia sclerotiorum, R. solani, Macrophomnia phaseolina and A. solani* from tomato fields area in Boushehr province south of Iran(KaramPur et al., 2004). Their experiments showed that the fungal isolated agents inhibit emergence and growth of broomrape stalks(KaramPur et al., 2004). In Nepal, when fungi were isolated from visibly infected plants, it was found that over 70% of the isolates were from the genus *Fusarium*. In Hungary, during trials to control *O. ramosa* with strains of *F. solani* and *F. oxysporum* isolated from the plant test, a single treatment achieved a mortality of over 90% in the first year and about 97% in the second, showing the capacity of these fungi to survive in the soil and to maintain or even enhance their virulence over time, especially in the case of *F.oxysporum* (Hodosy, 1981). Equally encouraging results were obtained in India against *O. cumana* (Bedi and Donchev, 1991)while in Egypt some fungi isolated from the fruit of *O. crenata*, including the fungus in question here, reduced seed viability in *O. crenata* by 21–85% . Very encouraging results were also achieved by Amsellem et al. (2001) who obtained two isolates, one of *F. arthrosporioides* and one of *F. oxysporum*, that parasitized plants of *O. ramosa*, *O. aegyptiaca* and *O. cernua* but not *O. cumana* nor the various crop plants tested: this result, reached through a series of artificial inoculations, was confirmed with an in-depth bio-molecular analysis (RFLP and RAPD) which showed the considerable genetic difference between these two isolates, and between these isolates and many other isolates of *Fusarium* spp. and formae specials of *F.*

oxysporum, including an isolate of *F. oxysporum* parasitizing *O. cumana*. Amsellem et al. (2001) also found that tomato plants, whose root apparatus had been treated with a conidial suspension and hyphal fragments of the two isolates and had then been planted in soil infested with *O. ramosa*, were completely protected for 6 weeks (Amsellem et al., 2001).*F. oxysporum*, together with *F. semitectum*, was also reported as a possible pest control agent for Cuscuta (Stojanovic and Boric, 1981).Other *Fusarium* spp. have also been indicated as possible control agents of *Orobanche*. Bozokov and Zouzmanova (1994) tested *F. lateritium* (*Gibberella baccata*) against *O. ramosa* and *O. mutelii* on tobacco; they found that the fungus was more effective when it was distributed as conidia or in the plant's hypogeal phase, especially between germination and tubercle formation, and that it remained effective longer if it was distributed in irrigation water(Bozoukov and Kouzmanova, 1993). Besides the species named, only a few other fungi are reported to be parasites on *Orobanche* spp. and hence are potential pest control agents; the list seems limited to *Botrytis cinerea* on *O. fasciculate* near Washington (USA) (Shaw, 1973; Farr et al., 1989);, *Thielaviopsis basicola* (=*Chalara elegans*) on *O. ramosa* in the Ukraine (Popova, 1929), *Colletotrichum lagenarium* on *O. aegyptiaca* in eastern Europe (Stojanovic and Boric, 1981), and *Ulocladium atrum* isolated, together with a *Fusarium* sp. and an *Alternaria* sp., from plants of *O. crenata* and *O. minor* picked in Syria, Morocco and France, and which destroyed the tubercles in the soil or the sprouts which had just emerged (Linke et al., 1992). Considerable attention is currently being focused on the micromycetes that control pests and parasites biologically (Sauerborn, 1993), although recent research undertaken in Hungary has shown the scant efficacy of such methods of control with certain plant-pathogen associations, including *Fusarium* spp. and *Orobanche* spp(Béres et al., 2000). .In examining whether certain fungi will control weeds or plant parasites, two different approaches can be followed: either a particularly efficacious isolate can be distributed in the field and its effect optimised; or alternatively a better understanding of the relationship between a crop plant and its parasite(Müller-Schärer et al., 2000). Moreover, we need to bear in mind that, despite the promise offered by *F. oxysporum* to control *O. ramosa*, the fungus may yet be found to be harmful to some crop species, making it impossible to use it as a biocontrol agent (Murasheva, 1995). Our survey produced encouraging data on the possibility of using *F. solani* in the biological control of *O. ramosa* in the not too distant future.

REFERENCES

Abouzeid MA, Boari A, Zonno M, Vurro M, Evidente A (2004). Toxicity profiles of potential biocontrol agents of *Orobanche ramosa*. Weed Science 52 (3):326-332.

Amsellem Z, Kleifeld Y, Kerenyi Z, Hornok L, Goldwasser Y, Gressel J (2001). Isolation, identification, and activity of mycoherbicidal pathogens from juvenile broomrape plants. Biological Control 21 (3):274-284. doi:10.1006/bcon.2001.0934

Andolfi A, Boari A, Evidente A, Vurro M (2005). Metabolites inhibiting germination of *Orobanche ramosa* seeds produced by *Myrothecium verrucaria* and *Fusarium compactum*. Journal of Agricultural and Food Chemistry 53 (5):1598-1603. doi:10.1021/jf048339i

Bedi JS, Donchev N (1991). Results of mycoherbicide control of sunflower broomrape (*Orobanche cumana* Wallr.) under glasshouse and feld conditions. In: Ransom JK, Musselman LJ, Worsham AD, Parker C (eds) 5th International Symposium on Parasitic Weeds, Nairobi, Kenya, 1991. pp 76-82

Béres I, Fischl G, Mikulas J (2000). Biological weed control with fungal pathogens in Hungary. Paper presented at the 20th German Conference on Weed Biology and Weed Control, Stuttgart-Hohenheim, Germany, 14–16 March

Bozoukov H, Kouzmanova I (1993). Biological control of tobacco broomrape (*Orobanche* spp.) by means of some fungi of the genus *Fusarium*. Paper presented at the Third International Workshop on Orobanche and Related Stiga Research, Amsterdam, Netherlands, 8–12 November

Duafala T, Wilhelm S, Gold AH, Sagen J (1976). *Rhizoctonia* disease of broomrape, a possible biological control American Phytopathologist, 1976. p 272

Farr DF, Bills GF, Chamuris GP, Rossman AY (1989). Fungi on plants and plant products in the United States. APS Press, St., Paul, MN, USA,pp.

Galdames R, Diaz J (2010). Stem rot of branched broomrape (*Orobanche ramosa*) caused by *Sclerotium rolfsii* in Chile. Plant Disease 94 (10):1266-1266. doi:10.1094/pdis-05-10-0370

Ghotbi M, AminiDehghan M, Ghotbi M (2011). Effect of nutritional regime on expression of pathogenicity of *Fusarium oxysporum* against *Orobanche aegyptiaca* Pers. International Journal of AgriScience 1 (4):012-031.

Hadj Seyed Hadi M, Fazeli F, Taghi Darzi M, Shahmoradi R (2005.). Biological control of *Orobanche aegyptiaca* in tomato. Paper presented at the The Fourth World Congress on Allelopathy, NSW Australia, 21 - 26 August

Hasannejad S, Zad SJ, Alizade HM, Rahymian H (2006). The effects of *Fusarium oxysporum* on broomrape (*Orobanche egyptiaca*) seed germination. Communications in agricultural and applied biological sciences 71 (3 Pt B):1295-1299.

Hodosy S (1981). Biological control of broomrape, *Orobanche ramosa*, a tomato parasite. I. Occurence and adaptability of *Fusarium* species to control broomrape in Hungary. Zoldsegtermesztesi Kutato Intzel Bulletinje 1421-29.

KaramPur F, Fasihi M, Heydari A-L. (2004). Identification of fungal pathogenic agents of broom rape on tomato fields. Tehran

Leslie JF, Summerell BA, Bullock S (2006). The Fusarium laboratory manual, vol 2. Vol 10. Wiley Online Library, pp.

Linke KH, Scheibel C, Saxena MC, Sauerborn J (1992). Fungi occurring on *Orobanche* spp. and their preliminary evaluation for *Orobanche* control. *Tropical Pest Management* 38 (2):127-130.

Mazaheri A, Ershad J (1995). Reactions of different crops to *Fusarium solani* fungal pathogen of broomrape. Paper presented at the 12th Iranian Plant Protection Congress Karadj 2-7 September 1995

Mazaheri A, Vaziri M (1991). Studies on the chemical control of broomrape (*Orobanche* spp) in tobacco fields. 10th Plant Protection Congress of Iran Kerman 1-5 Sept 1991.

Mehrabi Kushki A (2006) Applaication of antagonists *Fusariun* in biological control of bromrape. Tarbiat Modares, Tehran, Iran.

Müller-Schärer H, Scheepens PC, Greaves MP (2000). Biological control of weeds in European crops: recentachievements and future work Weed Research 40 (1):83-98.

Müller-Stöver D, Kroschel J, Thomas H, Sauerborn J (2002). Chlamydospores of *Fusarium oxysporum* Schlecht f.sp. *orthoceras* (Appel & Wollenw.) Bilai as inoculum for wheat-flour - Kaolin granules to be used for the biological control of Orobanche cumana Wallr. European Journal of Plant Pathology 108 (3):221-228.

Murasheva VN (1995). Influence of *Fusarium oxysporum* var. *orthoceras* [Appel. et Wr.] Bilai toxic properties on its vitality in soil and pathogenicity). Mikologiya i Fitopatologiya 29 (4):53-58.

Popova AA (1929). Diseases of the Tobacco *Nicotiana rusticaL* Morbi Plantarum 18 (1-2):45-53.

Press M, Graves J (1995). Parasitic Plants. Malcolm C. Press.292 pp.

Sauerborn J (1993). Natural antagonists for parasitic weed control in agroecosystems. In: integrated weed management for sustainable agricultur. Paper presented at the Indian Society of Weed Science International Symposium, Hisar, India, 18–20 November

Sharma P, Rai PK, Siddiqui SA, Chauhan JS (2010). First report of *Fusarium* wilt in the broomrape parasite growing on *Brassica* spp. in India. Plant Disease 95 (1):75-75. doi:10.1094/pdis-07-10-0546

Shaw CG (1973). Host fungus index for the Pacific Northwest.1. Hosts Washington State Agricultural Experimental Station Bulletin 7651-121.

Stojanovic D, Boric B (1981). Prilog proucavanju mikoflore parazitnih cvetnica iz rodova *Cuscuta i Orobanche*. [Contribution to the study of the mycoflora of parasitic phanerogams from the genera Cuscuta and Orobanche]. Zastita Bilja 32 (2):135-145.

Thomas H, Heller A, Sauerborn J, Muller-Stover D (1999). *Fusarium oxysporum* f sp *orthoceras*, a potential mycoherbicide, parasitizes seeds of *Orobanche cumana* (Sunflower, Broomrape): a cytological study. Annals of Botany 83 (4):453-458. doi:10.1006/anbo.1998.0847

Thomas H, Sauerborn J, Millerller-Stover D, Ziegler A, Bedi JS, Kroschel J (1998). The potential of *Fusarium oxysporum* f. sp. *orthoceras* as a biological control agent for *Orobanche cumana* in sunfower. Biological Control 1341-48.

Zonno M, Vurro M (2002). Inhibition of germination of *Orobanche ramosa* seeds by *Fusarium* toxins. Phytoparasitica 30 (5):519-524. doi:10.1007/bf02979757