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PROSPECTS OF MYCOHERBICIDES FOR CONTROL OF BROOMRAPES (*OROBANCHE* SPP.) IN EGYPT

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Abstract: Broomrapes (*Orobanche* spp.) are important parasitic weeds of peas, faba bean and tomatoes and other winter crops in Egypt. They are widespread and are major factors limiting production of these crops. From an extensive survey of Egyptian soils naturally infested with broomrapes, 42 isolates of fungi belonging to genera of *Alternaria, Fusarium* and *Trichoderma* were identified as pathogens of broomrapes under laboratory and greenhouse conditions. Three isolates of *Trichoderma* spp. including *T. harzianum* T_1, *T. harzianum* T_3 and *T. viride* T_2 were further tested for control of *Orobanche* spp. in peas, faba bean and tomatoes under field conditions. Results of field studies showed that soil treatment with these three fungal agents alone or soil treatment with fungal agents plus aerial spray of glyphosate (50 ppm) was effective in reducing infection of broomrapes and increasing yields of peas, faba bean and tomatoes. The prospect of developing *T. harzianum* T_1, *T. harzianum* T_3 and *T. viride* T_2 as mycoherbicides for control of bromerapes of peas, faba bean and tomatoes in Egypt is discussed in this paper.

Key words: broomrapes, control, glyphosate, herbicide, mycoherbicide, *Orobanche* spp., parasitic weeds, *Trichoderma* spp.

INTRODUCTION

Weed infestation is a serious problem in agriculture and it was estimated to cause up to 10% of crop losses in developed countries and two or more times more in developing countries (Labrada *et al*. 1996). Broomrapes (*Orobanche* spp.), the phytoparasitic weeds, are obligate parasites which grow on roots of a broad range of broadleaf plants. Some species attack food and ornamental crops, causing considerable yield losses (Parker and Wilson 1986). These parasitic weeds are wide-spread in the Mediterranean region, South East Europe, North Africa and the Middle East (Parker 1986; Parker and Riches 1993). In Egypt broomrapes are widespread on winter crops and have become one of the most serious problems for production of these crops. They parasitize numerous food crops belonging to various families such as *Fatalae, Compositae, Solanaceae, Cruciferae, Graniaceae, Umbelliferae* (Abdel-Kader *et al*. 1996). Significant losses attributed to infection by *Orobanche* spp. may occur in agricultural crops. For example, *Orobanche crenata* Forsk is a permanent threat of winter legume crops in southern and eastern Spain, and in 1996 it destroyed 80% of the pea crop grown in the province of Seville (about 40.000 ha) causing an estimated loss of about 1.600 million Pesets (Garcia-Torres *et al*. 1998) . In heavily infested fields in Egypt, broomrape can cause total crop failure whereas the percentage of infection by *Orobanche* spp. could reach up to 90-100% (Anonymous 1994). At different cultivated locations, in Egypt, the actual area infested with the broomrape and yield losses due to infection were estimated. In Behera province, the infested area was reported to be 65% of the total cultivated fields causing about 19 000 tons yield loss of winter crops (Zaitoun 1990). Moreover, the intensity of broomrape parasitic on faba bean plants in Minofia province was recorded to be 1–4 spikes per plant that caused 15–53% yield reduction (ARC 1998). The endemic spread of the parasite in the Nile of the Delta, estimated to cover about 18–86% of cultivated area caused 7–80% yield reduction (Korashi *et al*. 1996), the exceptionally long survival of seeds in soil along with the high susceptibility of different host cultivars rendered the issue of control of a paramount importance.

Parasitic weeds of the genus *Orobanche* (broomrapes) have a tremendous impact on world agriculture. Unlike other weeds they are devoid of leaves and are totally dependent on a host plant for nourishment. In the 20th century the means for Orobanche control included field sanitation, weeding, soil fumigation and solarization, selection and breeding of resistant crops, use of trap and catch crops and biological control. Control measures by cultural practices and chemical herbicides were also tried by many investigators, however, none of them were practical and economical for control of broomrapes in the majority of crops (Pieterse *et al*. 1992; Petzoldt *et al*. 1993; Epple and Norris 1996; Raju 1996). By the end of the 20th century, some herbicides proved relatively effective in

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certain crops. Glyphosate application was recommended by several researchers, for large scale application in faba bean fields, under heavy infestation *Orobanche* spp. The application of glyphosate at the rate of 0.07 kg a.s./ha during the root attachment stage was efficient for controlling *O. foatida* on faba bean. Periodical using of glyphosate at different doses to tobacco fields reduced Orobanche emergence by 50% (Raju 1996). In Egypt three times, glyphosate foliar application at the beginning of flowering of faba bean at three-week intervals reduced Orobanche incidence by 97-100% and increased seed yield by 34-124% (ICARDA 1983). The use of herbicides, however, must be tested against different crops and *Orobanche* species.

Application of glyphosate post-emergence at reduced rates have been successfully used in some crops, such as faba bean and sunflower. In this regard, in pot and field studies chlorsulfuron 75% WP (Eastnine Sanitary Ware Company, China) glyphosate [Touchdown, *N*-(phosphonomethyl) glycine], trimesium; Syngenta, Basel, Switzerland) and imazaquin **(**BASF, Germany) were applied 4-5 weeks after tomato transplanting, at the early flowering stage, when broomrape started to develop underground attachments to the host plant roots. The number of emerged broomrape shoots and underground attachments were less affected by herbicide treatments, suggesting that herbicides suppress the growth of broomrape rather than kill its underground organs (Korashi *et al.* 1996).

Biological control could play a major role in the management of broomrapes in crop production depending on the potential of natural enemies as bioagents. Promising mycoherbicidal microorganisms for control of parasitic weeds *in vitro* and/or *in vivo* have been reported by many investigators (Al-Menoufi 1986; Murasheva 1995; Abdel-Kader *et al.* 1998; Gronwald *et al.* 1998). Many investigators used *Fusarium oxysporum* f. sp. *orthoceras*, a pathogen of *Orobanche* spp., as a potential agent for biological control of the root parasitic weeds *Orobanche cumana* Wall., *O. ramosa* and *O. aegyptiaca* in sunflower, tobacco and other crops. The fungus attacks underground tissues of *Orobanche* spp. such as young plants, tubercles and germ tubes of seeds. *Fusarium oxysporum* f. sp. *orthoceras* has a potential for control of sunflower broomrape (*O. cumana*) as soil application of *F. oxysporum* f. sp. *orthoceras* into soil reduced seed germination of *O. cumana* and increased seed yield of sunflower (Bedi *et al.* 1991, 1994; Thomas *et al.* 1998). This fungus penetrates seeds and destroyed seed contents of broomrape, thereby reduced seed bank of this parasitic weed in soil (Thomas *et al.* 1999 a, b). Bozoukov and Kouzmnova (1994) recorded another example the use of *Fusarium lateritium* for control of tobacco broomrape (*O. ramosa* and *O. aegyptiaca*). The application of *F. lateritium* to the soil as mycelia obtained from anaerobic fermentation for up to 48 hr, chlamydospores for up to 80 hr or conidia obtained through anaerobic fermentation on sterilized barley caused a reduction of broomrape incidence by 62-68%. A recent report indicated that the application of strains of *Fusarium* sp., *F. oxysporum* or *F. arthrosporioides* by treatment of tomato seeds or seedlings prior to transplanting or by soil-drenching was effective in controlling nodding/drooping (*O. cernua* Loefi.) and branched broomrape of tomato and other vegetable crops (Amselem *et al.* 2001). Roots of tomato plants dipped in spore suspension of these Fusarium isolates and planted in broomrape-infested soil were protected against invasion for 6 weeks, meanwhile nearly 90% control of broomrape infection could be achieved by drenching transplant soil with these isolates.

So far, Orobanche research in the last twenty years has contributed to better understanding of biology, host-parasite relationships and control of broomrapes by chemical herbicides and microorganisms. These research achievements should be exploited for the development of novel control methods. Maintaining an equilibrium between the parasite population and its hosts by constant supply of a biocontrol agent that will keep Orobanche seed production to a minimum.

The present research aimed at developing alternative weed management strategies, including herbicidal and biological control. An integrated approach combining the herbicide glyphosate (Roundup) at low rates and soil infestation with the candidates mycoherbicides as control measures could allow a long-term solution of the Orobanche problem.

MATERIALS AND METHODS

Survey of Egyptian soils for fungal pathogens of broomrapes

Five Egyptian counties representing North, Middle and South Egypt were surveyed for the existence of natural infestation of broomrapes in different crops (Abdel-Kader *et al.* 1996). Samples of both healthy and broomrape infected various host plants including their rhizospheric soil were collected during the winter season of 1993-1994 and subjected to isolation of associated fungi according to the methods developed by Louw and Webly (1959). Total fungal count was followed according to Allen (1961) using Martin medium (Anonymous 1978). Fungal colonies were counted after 5-7 days of incubation at 25°C. Isolated fungi from the rhizosheric soil were purified and identified (Gilman 1957; Barnett and Hunter 1972). Pure fungal cultures were maintained on PDA slants (Anonymous 1978) at 5°C for further studies.

In vitro pathogenicity of fungi to *Orobanche* spp.

The isolated fungi were evaluated for their ability to parasitize *Orobanche* spp. under *in vitro* conditions (Abdel-Kader *et al.* 1996). Isolated pure fungal cultures (Table 1) were divided into three groups according to geographical samples, i.e. North Egypt (Beheira, Dakahlia and Gharbia provinces), Middle Egypt (Giza and Bani-Seuf province) and South Egypt (Assiut province). Juveniles of underground stage of both *O. crenata* and *O. ramose* obtained from infected faba bean and tomato plants at heavily infested fields were used in this study. Selected apparently healthy undamaged juveniles were surface sterilized with 1% sodium hypochlorite solution, washed in sterilized water and air dried onto filter paper, then placed on one side of Petri dishes containing PDA medium, while the opposite side was inoculated with each of the tested fungal isolate. Three plates were used as replicates for

each tested fungus. All plates were incubated at 25°C for one week and then examined. The ability of tested fungal isolates to invade and colonize broomrape tissues was determined (Kiraly *et al.* 1974) as high (damaged area > 50%), moderate (damaged area between 25–50%), weak (damaged area < 50%) and no infection (undamaged *Orobanche* tissues), with respect to the percentage of damaged tissues area resulting from *Orobanche* reaction to different fungi inoculated onto the medium.

Greenhouse experiments:

Control of broomrape of faba bean by fungi

The fungal isolates that proved their ability to invade the juvenile *Orobanche* tissues under *in vitro* conditions were used in greenhouse test. Pot experiment was carried out to evaluate the pathogenic ability of tested fungi to parasitize either *Orobanche* or faba bean as a model for host plants. Faba bean seeds (Giza 3 cv.) were sown in pots containing naturally heavily contaminated soil with a seed bank of *O. crenata* artificially infested individually with the tested fungi (at the rate of 5% w/w). Five seeds were sown per pot (25 cm-Diameter) and five pots were used as replicates for each particular treatment. Fungal infection on faba bean was observed throughout the growth period from sowing date until flowering stage (100 day old). The reduction percent of emerged juvenile *O. crenata* above the soil surface and their infection in each fungal treatment individually was calculated in comparison with control (un-infested soil with tested fungi). The same pots with the same treatments were used for two successive seasons during 1995-1997 in order to evaluate the long term effect of introduced fungi on either faba bean or broomrape seed bank in soil.

Field experiments:

Control of *Orobanche* spp. by fungi

Further studies, looking for cost-effective applicable and technique to gain the highest effect of the present fungi against broomrape infection, successive experiments were carried out under field conditions using only the candidates of fungal isolates which proved themselves as promising mycoherbicides. Three candidate isolates of fungi including *T. harzianum* and *T. viride* given a code of T_1; T_3 and T_2 respectively were selected from the laboratory and greenhouse experiments and used for field experiments to evaluate their potential as mycoherbicides for controlling of broomrapes on pea, faba bean and tomato plants. Although some isolates of *Alternaria* spp. and *Fusarium* spp. were also proved as pathogenic on *Orobanche* spp. in the laboratory and greenhouse tests, they were not used in the field trials due to a possibility of being pathogens of different host crops.

A series of successive field trails for evaluating different approaches to control of broomrape infection was carried out in a field naturally heavily infested with *Orobanche* spp. sown at Al-Aiat territory, Giza province. All *Orobanche* host plants, i.e. pea, faba bean, tomato which had been grown in this field were severely damaged by infections causing annual loss record about 50% during the last ten previous seasons (Abdel-Kader *et al.* 1996, 1998).

The following procedures were carried out in all field trails. The experiments consisted of four treatments, including *T. harzianum* (T_1), *T. harzianum* (T_3), *T. viride* (T_2) and untreated control. Treatments were arranged in a completely randomized block design with three replicates (plots) for each particular treatment. The dimensions of each plot were 25 x 7 m (0.02 ha). Pea, faba bean and tomato were used as broomrape host plants. Methods of traditional agricultural practices were followed from sowing until the end of the growing season.

The broomrape infection was expressed as percentage of the above ground visual flowering stalks of *Orobanche* around the host plants (disease incidence) or their numbers attached to the roots of host plants (disease severity). The percentage of broomrape infection was calculated as numbers of infected cultivated host plants with *Orobanche* spp. in relative to the total number of plants in the experimental plot. To calculate the severity of broomrape infection, infected host plants were classified into five categories according to the number of attached *Orobanche* juveniles, i.e. one, two, three, four and more than four broomrape juveniles per host plant roots, and the severity index for each plot was calculated using the formula described by Chastanger and Ogawa (1979) as follows:

$$D.S. = \frac{\sum (n \times c)}{N}$$

where: D.S. = Disease severity
n = Number of infected plants per category
C = Category number
N = Total examinal plants

At harvest time the obtained yield of each cultivated crop was determined for each particular treatment as average weight of produced yield per experimental plot (0.02 ha).

The first preliminary field trail was carried out in pea field to get superior fungal strains that are capable to control the parasitic weeds and reduce or eliminate, if possible, the necessity of using herbicides under field conditions (Abdel-Kader 1999). Mycoherbicides inocula of *T. harzianum* (T_1 & T_3) and *T. viride* (T_2) were prepared as spore suspensions ($5x10^6$ conidia/ml) and drenched to pea plant. There were seven sprays each at the rate of 100 l per 4200 m^2 (0.42 ha) with 15 days intervals starting 15 days after pea seedlings' emergence.

Artificially introduced fungi to the soil face different factors, *i.e.* physical and chemical characters, pH, salinity, soil moisture, organic compounds, competition with other soil microorganisms, etc. which affect their survival in soil. Therefore, they have to overcome undesirable conditions to success and establish their inoculum potential.

To enhance survival and efficacy of mycoherbicidal agents, different application methods for *T. harzinurn* (T_1), *T. harzianum* (T_3) and *T. viride* (T_2) were compared for control of broomrape infection on peas in the field. The application methods were: (i) seed treatment, (ii) foliar spray, (iii) seed treatment plus foliar spray, (iv) soil drenching and (v) soil drenching plus foliar spray (Abdel-Kader and El-Mougy 2001). Pea seeds were immersed for one hour before planting in the prepared conidial suspension of

each particular mycoherbicidal isolate, while foliar spray was followed as described earlier (Abdel-Kader 1999). Mycoherbicidal inocula grown for 14 days on sand-barley medium (1:1, w:w and 40% water) were used for soil drench. Mycoherbicidal inoculum at the rate of 120 g/m^2 (Abdel-Kader 1997) was incorporated in the top 20 cm of soil surface at planting row sites one week before sowing date, relevant to the specific treatment.

Integrated control of broomrapes

Field studies were conducted to investigate the integrated methods of biological control and chemical control for management of broomrapes in faba bean (Abdel-Kader and El-Mougy 2002). The herbicide glyphosate [N-(phosphonomethyle) glycine] was recommended for control of broomrape in different crops (Kharat *et al.* 1994; Raju 1996) and it was tried for control of broomrape in faba bean fields in Egypt during 1980-1983 (ICARDA 1983).

The influence of a chemical herbicides and mycoherbicides *T. harzinum* (T_1), *T. harzianum* (T_3) and *T. viride* (T_2) introduced to the soil was evaluated. The inhibitory effect of glyphosate on the mycelial radial growth of the used *Trichoderma* spp. was also evaluated *in vitro*. Glyphosate (36%) at 15 concentrations from 0 up to 140 ppm with a constant increase by 10 ppm was tested. Different volumes of glyphosate stock solution were added to the SM medium before solidification, to give the desired concentration, then poured at approximately equal voulmes to 20 ml/plate. A set of Petri dishes containing glyphosate-free medium was kept as check. Disks (5 mm in diameter) from 7 days old cultures were placed in the middle of each dish and five replicates for each particular treatment as check were used. Plates were incubated for 7 days at 25 ±1°C, then examined. The percentage of growth reduction of the fungi tested in different treatments in relation to the control was calculated. Spores produced were counted just after the latest determination of the radial growth on solid medium. Fungal spores of each particular treatment were suspended in of 100 ml distilled water and counting was made using a heamacytometer slide. The average number of spores was calculated per mm^2 of fungal growth in each particular treatment.

Integration between mycoherbicides and glyphosate treatments for enhancing efficacy of control of *O. crenata* was investigated in faba bean fields. For the purpose of increasing the inoculum potential of mycoherbicides, the three fungi *T. harzianum* (T_1; T_3) and *T. viride* (T_2), were introduced to the soil in a form of compost. Inocula grown for 15 days on sand barely medium (1:1, w:w and 40% water) were used individually at the rate of 10% (w:w) for inoculation the prepared compost (containing plant material wastes) and thoroughly mixed. Regular mixing every 10 days was made for 30 days with adjustment of the moisture content to about 40%, before the use for soil drenching.

The inoculated compost substrate was separately introduced into the soil by drenching and mixing with the top soil for up to the depth of 20 cm at the rate of one m^3 per plot (175 m^2). The mycoherbicide-inoculated soils were irrigated and left for one week before sowing of faba bean.

The applied treatments were: (i) mycobiocides (*T. harzianum* T_1 ; T_3 and *T. viride* T_2) applied to the soil by soil drenching, (ii) foliar spray of glyphosate (7L per plot), and (iii) soil drench of mycoherbicides plus foliar spray with glyphosate. Faba bean seeds cv. Giza 3 were sown in all plots (each comprised 12 rows with 30 seed bed/row), and traditional agricultural practices were followed. Foliar spray with the herbicide glyphosate (50 ppm) was applied to developed plants at 30 and 40 days after sowing.

The percentage of faba bean plants infected by *O. crenata* as well as the intensity of attack was estimated at the end of the growing season. All treatments either mycobiocidal or herbicidal resulted in a significant decrease in *O. crenata* incidence.

Further integration study between mycobiocides and the herbicide glyphosate was evaluated for efficacy in controlling the incidence of broomrape in a tomato field naturally infested with *O. ramose* seed bank (Abdel-Kader and El-Mougy 2007). The applied treatments were: (1) transplants separately inoculated with mycoherbicide (*T. harzianum* T_1; T_3 and *T. viride* T_2); (2) inoculated transplants plus foliar spray with glyphosate; (3) uninoculated transplants plus foliar spray with glyphosate; (4) uninoculated transplants. Tomato seeds cv. GS_{12} (obtained from Horticulture Institute, Ministry of Agriculture, Egypt) were sown into polystyrene foam cells containing peat-moss soil. One week after seed germination, They were irrigated three times (once a week) with a growth suspension ($5x10^5$ cfu/ml) of each mycoherbicide at the rate of 1 L/tray (polystyrene foam containing 86 cells). 40 days after sowing, tomato seedlings were transplanted to the experimental field and traditional agricultural practices were followed until the end of the growing season. Ten days after transplanting, tomato plants were sprayed with glyphosate (50 ppm). The percentage of plants infection by *O. ramosa* and the intensity of attack as well as the obtained yield were estimated at the end of the growing season.

Statistical analysis

Obtained data were statistically analyzed according to Steel and Torrie (1980). Data were submitted to analysis of variance. The significance of effect of the used treatments was determined at $p = 0.05$. Treatment means were separated by least significant difference (LSD).

RESULTS AND DISCUSSION

Survey of Egyptian soils for fungal pathogens of broomrapes

Quantitative and qualitative differences were received for isolated fungi (Table 1) under infected and uninfected conditions of *Orobanche* spp. underground young tissues, and they represented different fungal genera of *Alternaria, Aspergillus, Cladosporium, Fusarium, Mucer, Penicillium, Rhizoctonia, Rhizopus, Synchetrium, Trichothecium, Trichoderma* in addition to other unknown fungi. Such fungi were also isolated from the soil or diseased *Orobanche* spp. (Talsakh Yan and Grigoryan 1978; Al-Menoufi 1986). The present results revealed that both the presence and distribution of a given fungal genus in the rhizosphere of

Table 1. Frequency of occurring fungi in the rhizosphere of various plants under broomrape infestation conditions throughout Egypt

Surveyed country[A]	province	Broomrape infection	The highest counts of fungal genera	The main host plant
North Egypt	Beheira	infected	*Fusarium, Aspergillius, Penicillium, Ttrichoderma*	pea/tomato/ bean
		healthy	*Trichoderma, Alternaria, Trichothecium, Rhizopus*	pea/tomato/bean
	Gharbia	infected	*Rhizoctonia, Mucor, Aspergillius, Synchytrium*	cabbage/tomato/ cauliflower
		healthy	*Trichoderma, Alternaria, Penicillium, Rhizoctonia*	cabbage/tomato/ cauliflower
	Dakahlia	infected	*Cladosporium, Fusarium, Alternaria, Rhizopus*	faba bean/pea/bean
		healthy	*Trichoderma, Cladosporium, Fusarium, Synchetrium*	faba bean/pea/bean
Middle Egypt	Giza	infected	*Alternaria, Fusarium, Penicillium, Rhizoctonia*	faba bean/pea/tomato
		healthy	*Trichoderma, Alternaria, Rhizopus, Penicillium*	faba bean/pea/tomato
	Bani-Seuf	infected	*Cladosporium, Synchytrium, Penicillium, Trichothecium*	faba bean/pea/tomato
		healthy	*Trichoderma, Trichothecium, Alternaria, Rhizopus*	faba bean/pea/tomato
South Egypt	Assiut	infected	*Alternaria, Rhizoctonia, Penicillium, Trichothecium*	chamomile/geranium/faba bean
		healthy	*Trichoderma, Cladosporium, Fusarium, Mucor*	chamomile/geranium/faba bean

[A] survey was carried out during the winter growing season 1993-1994

Orobanche infested and uninfested plants are correlated host, location, host growth stages, environmental conditions and broomrape infestation. The decomposable root debris and root exudates that supply fungi with sources of nutrients can facilitate the damage of roots by increasing the activity of saprophytic fungi.

The tested fungal isolates varied in their pathogenicity on broomrapes. Among 487 isolates of fungi tested, only 42 isolates belonging to genera of *Alternaria, Fusarium* and *Trichoderma* showed severe and moderate infection and colonization of broomrape tissues. No damage was produced by species of *Mucor*, *Rhizopus*, *Cladosporium*, *Synchytrium* and *Trichothecium* and phycomycetes. The parasitism could be attributed to defense mechanisms of broomrape or genetic variations of fungal isolates. In a similar study using 44 isolates of fungi belonging to genera of *Alternaria, Gliocladium, Fusarium* and *Sclerotinia,* only 9 isolates belonging to *Fusarium* spp. and *Sclerotinia* spp. produced severe rotting of broomrape tissues (Al-Menoufi 1986).

The variation in broomrape reaction to the highly pathogenic fungal isolates suggested the possibility of testing these fungi as potential mycoherbicides. To achieve this purpose the parasitism of those promising fungi on either faba bean as host plant or *O. crenata* as a main target was evaluated under greenhouse conditions.

Greenhouse experiments

Control of broomrape of faba bean by fungi

Results (Table 2) revealed interesting behaviour for the parasitic ability of the forty two tested soilborne fungi to *O. crenata*, while they failed to attack the host plant (faba bean) at pre- and post-emergence stages. Some fungal isolates belonging to genera of *Alternaria, Fusarium* and *Trichoderma* could attack seeds or germinated seeds of *O. crenata* and growing tubercles below soil surface, while they had a weak effect or even could not attack aboveground growing Orobanche juveniles tissues.

The opposite trend was observed with another set of fungal isolates of *Alternaria, Fusarium*. Only certain isolates of *Trichoderma* and *Fusarium* showed high aggressiveness to parasitize *O. crenata*. They attacked *O. crenata* juveniles before and after emergence above soil surface resulting in high reduction by 40-50% in counts and 80-100% infection (Table 2). The observed differences in the ability of tested soilborne fungi to attack *O. crenata* may be due to the fungal parasitism system as well as known soilborne pathogen groups causing damping-off, root rot, wilt, etc. in addition to the host defense mechanism which develop by growth period extend and became more tolerant or resistant to the attack by pathogenic fungi. This fact may explain the observed differences in the present results whereas, some of tested fungi (19 isolates) failed to attack *O. crenata* juveniles, while the rest of them (23 isolates) could invade juveniles causing the reduction in their counts and/or rot symptoms to others in infection percentages, although, all these fungi (42 isolates) proved to be invaders of Orobanche tissues under *in vitro* conditions (Abdel-Kader *et al.* 1996). These results are similar to pathogenicity of *Rhizoctonia solani, Fusarium lateritium, F. gibbosum, F. sambucium, F. orobanche* and *Verticillium microsporum* to juvenile plants, flowering stalk, flowers of *Orobanche* spp. (Barloy and Pelhate 1962; Duafala *et al.* 1975, 1976; Talsakh Yan and Grigoryan 1978). Although

Table 2. Influence of some soilborne fungi on reduction in counts and infection of juveniles of *O. crenata* in broomrape contaminated soil [A] cultivated with faba bean for two successive seasons under greenhouse conditions

Fungal isolates	Counts [B] (reduction %) of juveniles *O. crenata* in soil		Fungal infection [C] [%] of juveniles *O. crenata*	
	1995/96	1996/97	1995/96	1996/97
Alternaria sp. 8	53.3	40.0	0	0
Alternaria sp. 50	13.3	1.0	100	86.1
Fusarium sp. 10	50.0	37.5	0	0
Fusarium sp. 11	10.0	15.0	7.4	23.5
Fusarium sp. 17	50.0	37.5	0	0
Fusarium sp. 23	16.7	12.5	100	94.2
Fusarium sp. 35	50.0	37.5	46.7	52.0
Fusarium sp. 80	20.0	15.0	0	0
Fusarium sp. 115	16.7	12.5	100	83.3
Fusarium sp. 319	50.0	37.5	0	0
Fusarium sp. 320	50.0	35.0	80	84.6
Fusarium sp. A_1	10.0	1.3	0	0
Fusarium sp. A_5	33.3	25.0	100	90.0
Fusarium sp. A_{11}	33.3	27.5	35.0	44.8
Fusarium sp. A_{12}	40.0	30.0	100	89.2
Trichoderma sp. 2	35.3	40.0	100	100
Trichoderma sp. 3	33.3	27.5	0	0
Trichoderma sp. 40	6.7	17.5	0	0
Trichoderma sp. 77	36.7	30.0	100	92.6
Trichoderma sp. 148	33.3	25.0	0	0
Trichoderma sp. 190	33.3	25.0	0	0
Trichoderma sp. 194	33.3	30.0	0	0
Trichoderma sp. 205	50.0	40.0	100	100
Control – 1 [D]	0.0	0.0	0	0
Control – 2 [E]	0.0	0.0	0	0
LSD at 5% for:				
Fungal isolates (F)	16.8		18.4	
Season (S)	13.3		16.9	
Between (F) X (S)	31.2		26.4	

[A] unsterilized loamy soils either had a very large broomrape seed bank or free broomrape contamination obtained from fields at al-Aiat territory, Giza province, Egypt
[B] percentage of juveniles *O. crenata* counts calculated relatively to the numbers of emerged in control-1
[C] percentage of infected juveniles *O. crenata* calculated relatively to the number of emerged in each fungal treatment individually
[D] naturally infested soil with broomrape
[E] naturally uninfested soil with either Orobanche or tested fungi

juveniles of *O. crenata* in this study, were infected after emergence above the soil surface (attachment with host plant and their damage took place), they failed in seed formation. Moreover, the observed long term effect of introduced fungi lead to the conclution that *O. crenata* seed bank in contaminated soil will be decreased from one season to another depending on the parasitizing fungi introduced to the soil.

Field experiments

Control of *Orobanche* spp. by fungi

The effect of drenching the pea plant roots region with mycoherbicidal inocula for controlling *Orobanche* infection under field conditions is presented in Table 3. Results showed that all three isolates of *Trichoderma* spp. caused significant reduction of *Orobanche* infection on peas throughout the pea growing season. Moreover, a significant increase in the accumulative yield of green pea pods (Table 4) was recorded for the *Trichoderma*-treated plots, compared to the untreated controls.

The application of *T. harzianum* (T_1 & T_3) and *T. viride* (T_2) resulted in a significant reduction in pea infection (Table 3) with *O. crenata* in comparison with the control. The two species of *Trichoderma* differed significantly in their reaction on the infection with *O. crenata*, while no significance was observed between the two *T. harzianum* isolates T_1 and T_3. Data also showed that, *O. crenata* infection was decreased by 32.0; 30.0 and 61.2% in Trichoderma-treatments T_1, T_3 and T_2, respectively in comparison with the untreated control. Moreover, significant reduction in severity (Table 3) of infection by 58.0, 46.8 and 78.4% was recorded at the same above mentioned treatments. Furthermore, the preliminary study showed that the obtained reduction in the rate of invaded pea plants with *O. crenata* may be attributed to the high accumulative inoculum potential of the introduced *Trichoderma* spp. (T_1, T_2, T_3) into the root region throughout the growing season where they are expected to have a direct impact on already established *Orobanche* populations. On the other hand, the obtained reduction in severity of infection with *Orobance* (number of attached *O. crenata* juveniles per

Table 3. Effect of mycoherbicides' application to pea plants on incidence and severity of *O. crenata* infection under field conditions

Mycoherbicidal treatments [A]	Infection of pea plants with *O. crenata*			
	Incidence [B] [%]	Reduction [%]	Severity [C] [%]	Reduction [%]
T. harzianum (T_1)	24.0	32.0	39.0	58.0
T. harzianum (T_3)	24.7	30.0	50.3	46.8
T. viride (T_2)	13.7	61.2	20.3	78.4
Untreated	35.3	–	94.0	–
LSD at 5%	8.6	–	12.8	–

[A] mycoherbicides applied as spore suspensions drenched to pea plant root region (stems and the soil surface around them at the rate of 100 l per 4200 m^2 (0.42 ha)
[B] disease incidence was calculated as numbers of infected cultivated host plants with *Orobanche* sp. in relation to the total number of plants in the experimental plot
[C] disease severity was calculated as numbers of *Orobanche* juveniles that attached to the roots of pea plants using the formula described by Chastanger and Ogawa (1979)

Table 4. Effect of mycoherbicides' application on pea yield [A]

Mycoherbicidal treatments[B]	Yield characteristic			
	Green pods [ton/ha]	Increase [%]	1000 dry seeds /wt. [g]	Increase [%]
T. harzianum (T_1)	4.32	11.3	136.7	21.8
T. harzianum (T_3)	4.30	10.8	133.4	18.9
T. viride (T_2)	4.72	21.6	170.3	51.8
Untreated	3.88	–	112.2	–
LSD at 5%	6.4	–	1.6	–

[A] pea yield was determined as weight of green pods throughout the harvest period
[B] mycoherbicides applied as spore suspensions drenched to pea plant roots region (stems and the soil surface around them at the rate of 100 l per 4200 m^2 (0.04 ha)

plant) provide further evidence that the tested mycoherbicides (*Trichoderma* spp.) attack *O crenata* and can considerably reduce its intensity than compared to the native range recorded in untreated control. Trichoderma-treated pea plants were associated with 46.8% to 78.4% reduction in *O. crenata* juveniles per host plant. These results reveal that the application of *T. harzianum* and *T. viride* to field heavily infested with *O. crenata* successfully reduced pea plant infection. These results are confirmed by previously evaluation of the parasitic ability of *Trichoderma* spp. under laboratory and pot trails conditions (Abdel-Kader *et al.* 1996, 1998). They found that, *Trichoderma* spp. were able to colonize *Orobanche* tissues *in vitro* meanwhile, in pot trails they could attack *O. crenata* juveniles before and after emergence above soil surface resulting in high reduction of their count and host plant infection.

Reduction of infection with *O. crenata*, meaning the increase in numbers of healthy plants, resulted in high quantity of produced yield (Table 4). The total yield of pea plant, as green pods, in the Trichoderma-treated plots was increased over the control as much as 11.3, 10.8 and 21.6% for T_1, T_3 and T_2 treatments, respectively. The same trend was also observed concerning the weight of 1000 dry seeds. The influence of infection with *Orobanche* on yield production was reported by many investigators. Kharat *et al.* (1994) found that non-emergent *Orobanche* juveniles affect the growth and the seed yield of faba bean regardless of the number and biomass of emergent broomrapes. Also, the faba bean plants infected by *O. crenata* caused significant reduction in seed yield and seed quality. The reduction in yield and quality of faba bean was attributed to the nutrient transfer from host plant to the parasite especially during the pod formation period (Ibrahim 1997). The preliminary field study suggests that *T. harzinum* (T_1), *T. harzianum* (T_3) and *T. viride* (T_2) have a potential for development as mycoherbicides for control of *O. crenata* on pea plant**.** However, research on further improvement of applicable methods of *O. crenata* is needed. The present findings suggest the application of promising *Trichoderma* spp. as parasites against *O. crenata* under field conditions. The search for improving the methods and time of application is taken in consideration in the further study.

Evaluation of different approach of mycoherbicides' application for controlling *O. crenata* was carried out in pea field. Results showed that mycoherbicides applied as soil drenching followed by foliar spraying seven times throughout the growing season was the most effective method for control of *O. crenata* infection and severity on pea (Fig. 1, 2). The obtained reduction in invaded pea plants with *O. crenata* may be attributed to the high accumulative inoculum potential of the introduced *T. harzianum* (T_1), *T. harzianum* (T_3) and *T. viride* (T_2) into the root region, before pea sowing and throughout the growing season as well, where they are predicted to have a direct impact on already established *Orobanche* population. Similar explanation stated that *T. harzianum* applied as soil treatment was more effective than seed treatment in the control of root rot of bean caused by *Rhizoctonia solani*. In addition, the difference in efficacy between soil treatment and seed treatment could be due to differences in the level of initial inoculum of *T. harzianum* introduced into

the soil (Abdel-Kader 1997). Moreover, the soil treatment technique enables the introduced fungus to establish high population in the plant rhizosphere (Papavizas 1982). The present study showed that in all different methods of mycoherbicidal approach the infection of pea plants was associated with the reduction in *O. crenata* juveniles per pea host plant ranging between 33.1% up to 70.8%. These results were in a agreement with those previously reported (Abdel-Kader 1999). The reduction in infection by *O. crenata*, meaning the increase in the number of healthy plants, resulted in high quantity of produced yield (Fig. 3).

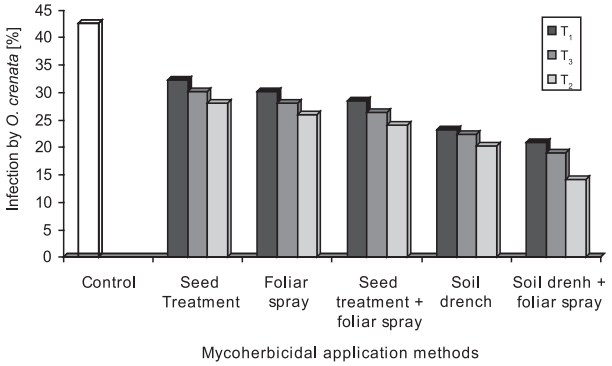

Fig. 1. Orobanche infection of pea plants in response to mycoherbicidal application

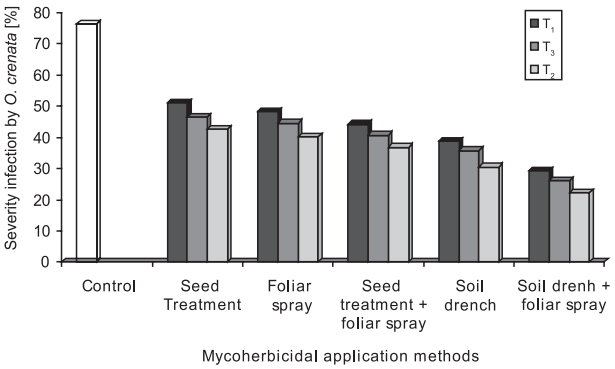

Fig. 2. Orobanche infection severity of pea plants in response to mycoherbicidal application

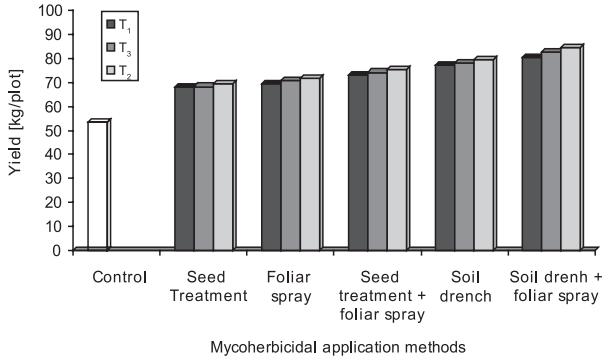

Fig. 3. Accumulative yield of pea plants in response to mycoherbicidal application

All the three mycoherbicidal treatments showed a significant increase of the pea yield obtained, as weight of green pods ranged between 68.0 up to 84.4 kg/plot in comparison with 53.6 kg/plot recorded in untreated control. The highest figures of pea yield (80.5, 82.6 and 84.4), were recorded in pea plots treated with *T. harzianum* (T_1), *T. harzianum* (T_3) and *T. viride* (T_2), respectively applied as soil drench before sowing followed by foliar spraying seven times throughout the growing season. Pea plots treated with mycoherbicides as soil drench only also showed significant increase in total yield over the control as much as much as 44.2, 45.8 and 48.5% for *T. harzinum* (T_1), *T. harzianum* (T_3) and *T. viride* (T_2), respectively. The increase in pea yield was less obvious for the application method of seed coating plus foliar spray with mycoherbicides and least obvious using the method of seed coating with mycoherbicides alone.

The same trend of treatment effects was also observed concerning the weight of 100 dry seeds. The influence of infection by *Orobanche* on yield production was also reported by many investigators (Kharat *et al.* 1994; Ibrahim 1997; Abdel-Kader 1999). Thus, the method of soil drenching and plant spray may be practical for field application of *T. harzianum* and *T. viride* to control *O. crenata* in peas. Development of more efficient application methods against *Orobanche* and/or improving the present ones in addition to improving the methods and time of application which was taken into consideration in the further work.

Integration between biological and chemical control for management of broomrapes' infection was investigated under laboratory and field conditions. The inhibitory effect of the herbicide glyphosate on the mycelial radial growth of the used *Trichoderma* spp. was evaluated *in vitro*. Results of an *in vitro* study showed that colony diameter of *T. harzianum* (T_1), *T. harzianum* (T_3) and *T. viride* (T_2) grown on agar media containing 70 ppm of glyphosate was reduced by 4.4, 6.7 and 5.6%, respectively (Table 5). Mycelial growth was completely suppressed by glyphosate at concentrations between 130 and 140 ppm. The effect of glyphosate on spore production of the tested fungi was more drastic than on the mycelial growth (Table 5). Complete inhibition of spore production was observed at concentration of 120 ppm for *T. viride* (T_2) and 130 ppm, for *T. harzianum* (T_1; T_3). Although no previous reports on the antimicrobial effect of glyphosate are available, the inhibitory effect of glyphosate on *T. harzianum* (T_1; T_3) and *T. viride* (T_2) observed in this study may be attributed to the biochemical activity of glyphosate which may inhibit 5-enolphyruvylshi-Kimate-3-phosphate (EPSPS), an enzyme of the aromatic acid biosynthetic pathway. This prevents synthesis of essential aromatic amino acids needed for protein biosynthesis in plants and fungal cells and spore formation (Worthing 1991; Parker and Riches 1993 ; Lolas 1994).

It is interesting to note that, the findings of present study indicated that glyphosate at the concentration higher than 60 ppm had a harmful effect on mycelial growth and spore production of *T. harzianum* (T_1; T_3) and *T. viride* (T_2) (Table 5). Therefore, the recommended (Anonymous 1998; 2000) rate of glyphosate at 135 ppm (75 ml a.s./100 l water) for weed control must be revised because of its potential harmful effect on natural beneficial soil microorganisms.

Moreover, integration of mycobiocidal and chemical treatments gave more protection to faba bean plants against broomrape invasion comparing with each treatment alone (Table 6). The mycobiocides applied as inoculated compost to the soil followed by two foliar sprays of

Table 5. Linear growth and spore production by mycobiocides in response to glyphosate at different concentrations *in vitro*

Glyphosate concentration [ppm]	Reduction (%) in mycobiocides' linear growth and spore production					
	T. harzianum (T_1)		*T. harzianum* (T_3)		*T. viride* (T_2)	
	growth reduction[A]	spores reduction[B]	growth reduction	spores reduction	growth reduction	spores reduction
check	0	0	0	0	0	0
10	0	0	0	0	0	0
20	0	0	0	0	0	0
30	0	0	0	0	0	0
40	0	0	0	0	0	0
50	0	0	0	0	0	0
60	0	0	0	0	0	0
70	4.4	7.2	6.7	8.3	5.6	6.4
80	53.4	61.3	64.5	69.2	55.6	63.2
90	66.6	72.4	70.3	78.6	60.2	69.7
100	74.5	86.2	76.7	86.4	71.2	78.6
110	80.0	91.3	87.8	92.3	85.6	93.2
120	89.9	96.2	96.7	95.2	95.6	100
130	97.6	100	98.2	100	100	100
140	100	100	100	100	100	100

[A] percentage of growth reduction of the fungi tested in different treatments was calculated relatively to the growth in the control
[B] the average number of spores was estimated per mm² of fungal growth in each particular treatment and percentage of their reduction in different treatments was calculated relatively to the spore production in the control

Table 6 . Incidence and intensity of broomrape in response to mycoherbicides' and herbicide treatments applied in faba bean field

Treatment		% broomrape incidence and severity of faba bean infection			
Mycobiocides	Glyphosate	incidence[A] [%]	reduction [%]	severity[B] [%]	reduction [%]
T. harzianum T_1	–	32.8	47.7	35.6	60.2
T. viride T_3	–	27.6	56.0	28.7	67.9
T. harzianum T_2	–	34.2	45.5	36.3	59.4
–	Once	57.3	8.6	53.5	40.2
–	Twice	46.5	25.8	40.6	54.6
T. harzianum T_1	Once	20.4	67.5	24.4	72.8
T. harzianum T_1	Twice	17.6	72.0	21.2	76.3
T. viride T_3	Once	10.7	83.0	8.3	90.8
T. viride T_3	Twice	8.3	86.8	4.6	94.9
T. harzianum T_2	Once	22.6	64.0	26.2	70.7
T. harzianum T_2	Twice	18.5	70.5	22.3	75.1
Untreated (check)		62.7	–	89.4	–
LSD at 5% for Mycoherbicide (M)		4.2		3.8	
Glyphosate (G)		6.1	–	2.4	–
Interaction between (M x G)		14.7		11.6	

[A] disease incidence was calculated as numbers of infected cultivated host plants with *Orobanche* sp. in relation to the total number of plants in the experimental plot
[B] disease severity was calculated as numbers of *Orobanche* juveniles that attached to the roots of faba bean plants using the formula described by Chastanger and Ogawa (1979)

glyphosate was the most effective treatment that resulted in the highest reduction in the incidence and severity of *O. crenata* on faba bean and the highest increase in crop seed yield (Tables 6, 7). These findings confirm previous reports (Abdel-Kader *et al.* 1998; Abdel-Kader 1999; Abdel-Kader and El-Mougy 2001) that *Trichoderma* spp. could attack juveniles of *Orobanche* spp. before and after emergence above soil surface, resulting in a high reduction in incidence of broomrapes. The field study demonstrates that the method of application consisting of the combination of soil drenching with *T. harzianum* (T_1; T_3) and *T. viride* (T_2) and plant spray with glyphosate (50 ppm) may be useful for controlling of broomrape on faba bean. The observed reduction in faba bean invasion with *O. crenata* may be attributed to the high amount inocula and the exponential increase of *Trichoderma* spp. (T_1; T_2 and T_3) established in the vicinity of the seed bed prior to planting and the root region thereafter.

Similar results were reported for *Fusarium oxysporum* f.sp. *orthoceras* incontrolling broomrape (*Orobanche* spp.)

Table 7. Yield of faba bean plants following application of mycoherbicides' and herbicide treatments in the field naturally infested with *O. crenata*

Treatment		Yield characteristics			
Mycobiocides	Glyphosate	yield ton/ha	increase [%]	1000 dry seeds wt. [g]	increase [%]
T. harzianum T_1	–	0.69	38.0	436	18.9
T. viride T_3	–	0.73	64.0	444	20.9
T. harzianum T_2	–	0.69	38.0	416	13.3
–	once	0.59	18.0	381	3.8
–	twice	0.61	22.0	392	6.8
T_1	once	0.74	48.0	497	35.4
T_1	twice	0.79	58.0	542	47.6
T_3	once	0.77	54.0	531	44.6
T_3	twice	0.81	62.0	584	59.1
T_2	once	0.73	46.0	488	32.9
T_2	twice	0.77	54.0	525	43.0
Untreated (check)		0.50	–	367	–
LSD at 5% for					
Mycoherbicide (M)		0.03		24.3	
Glyphosate (G)		N.S.	–	2.1	–
Interaction between (M x G)		0.02		4.3	

Table 8. Tomato infection with *Orobanche ramosa* in response to mycoherbicidal and herbicidal treatments under field conditions

Application methods	Mycoherbicides	Infection of tomato plants with *O. ramose*			
		incidence [%]	reduction [%]	severity [%]	reduction [%]
Transplants' treatment [A]	*T. harzianum* T_1	29.4	50.8	39.4	50.3
	T. harzianum T_3	27.6	53.8	36.3	54.3
	T. viride T_2	31.2	47.8	35.6	55.2
Transplants' treatment + Foliar spray [B]	*T. harzianum* T_1	21.3	64.3	31.4	60.5
	T. harzianum T_3	24.4	59.2	29.8	62.5
	T. viride T_2	26.2	56.2	28.6	64.0
Foliar spray	–	41.8	30.1	68.7	13.4
Untreated control		59.8	–	79.4	–
LSD at 5% Mycoherbicides (M)		1.2		1.1	
Glyphosate (G)		1.7	–	1.6	–
Interaction between (M x G)		3.4		5.3	

[A]tomato seeds were sown into polystyrene foam cells containing peat-moss soil. One week after seed germination, polystyrene foam cells were irrigated three times (once a week) with spore suspensions ($5x10^5$ cfu/ml) of each mycoherbicide *T. harzianum* (T_1 & T_3) and *T. viride* (T_2) at the rate of 1 liter/tray
[B] ten days after transplanting, tomato plants were sprayed with glyphosate (50 ppm)

in different crops (Bedi *et al.* 1991, 1994; Bozoukov and Kouzmanova 1994; Thomas *et al.* 1999a, b). In this regard, Murasheva (1995); Murasheva and Sizova (1995) reported that the use of *Fusarium oxysporum* var. *orthoceras* as a mycobiocide being not recommended due to certain pathological and toxicological aspects of some strains to sunflower, tomato and wheat crops. In the present study, the observed reduction in faba bean invasion with *O. crenata* may be attributed to the high use and the exponential increase of *Trichoderma* spp. (T_1; T_2 and T_3) established in the vicinity of the seed bed prior to planting and the root region thereafter.

The systemic action of glyphosate was considered in this work, and the application 30 and 40 days after sowing was tested. The latter periods were assumed suitable for the attachment and establishment of broomrape tubercles to the host plant (Worthing 1991).

Obtained in the present work results indicate that *Trichoderma viride* (T_3) plus double glyphosate spraying gave reasonable level of protection against *O. crenata* as expressed by high yield production. The present field study demonstrates that the method of application of *T. harzianum* and *T. viride* as soil drench and plant spray with glyphosate may be acceptable against broomrape infection. It could be concluded that the amount of introduced mycobiocides might be decreased by adapting a method of application for each crop and agricultural practices considering the contamination level of the soil with broomrape seeds. Studying the ecology and survival of Trichoderma in the field may lead to the development

Table 9. Tomato yield in response to mycoherbicide and herbicide application under field conditions

Application methods	Mycoherbicides	Av. accumulated yield [ton/ha]	% yield increase
Transplants' treatment[A]	*T. harzianum* T_1	8.32	124.7
	T. harzianum T_3	8.50	129.7
	T. viride T_2	7.79	115.3
Transplants' treatment + Foliar spray[B]	*T. harzianum* T_1	7.36	98.8
	T. harzianum T_3	7.68	107.4
	T. viride T_2	7.35	98.6
Foliar spray	–	5.53	49.4
Untreated control		3.70	–
LSD at 5%	Mycoherbicides (M)	0.46	
	Glyphosate (G)	0.72	–
	Interaction between (M x G)	0.12	

[A] tomato seeds were sown into polystyrene foam cells containing peat-moss soil. One week after seed germination, polystyrene foam cells were irrigated three times (once a week) with spore suspensions ($5x10^5$ cfu/ml) of each mycoherbicide *T. harzianum* (T_1 & T_3) and *T. viride* (T_2) at the rate of 1 liter/tray
[B] ten days after transplanting, tomato plants were sprayed with glyphosate herbicide (50 ppm)

of more efficient procedures against *Orobanche* and/or to development of the present ones. The time of application also must be considered with great concern.

Integration between mycoherbicidal and herbicidal approaches resulted in a significant decrease in *O. ramosa* infection. Moreover, the integrated treatment gave a superior protection to tomato plants against branched broomrape invasion comparing with each treatment alone. The two species of Trichoderma, significantly differed in their action on broomrape incidence either when applied alone or combined with glyphosate spraying, while no significant differences were observed between T_1 and T_3 isolates of *T. harzianum*. This approach caused the reduction in *O. ramosa* infection (Table 8) recorded as 50.8; 53.8 and 47.8% in Trichoderma treatments T_1; T_3 and T_2, respectively, over the untreated check. These records increased up to 64.3; 59.2 and 56.2%, respectively, for the corresponding treatment with glyphosate. However, the lowest effect was observed in tomato sprayed with glyphosate only. These treatments showed the reduction in *O. ramose* incidence by 30.1%. This low effect may be attributed to the use of glyphosate at 50 ppm concentration which is lower than the recommended dose of application being 135 ppm (Anonymous 1998, 2000). The used dose was suggested to avoid the harmful effect of a greater glyphosate concentration on the growth of mycoherbicide fungi (Abdel-Kader and El-Mougy 2002).

The effect of mycoherbicide and herbicide application on tomato yield was also observed. The reduction in infection by *O. ramosa*, meaning the increase of number of healthy plants, resulted in high yield (Table 9). All mycoherbicidal treatments showed a significant increase of tomato yield, between 128.7 up to 134.4 kg/plot in comparison with 64.8 kg/plot recorded in untreated control. The highest figures of tomato yield, (98.6 ; 98.8 and 107.4%), were recorded in tomato plots treated with *Trichoderma* spp. T_1, T_3 and T_2, respectively as soil drench before sowing followed by foliar spraying throughout the growing season. Tomato plots treated with mycoherbicides as soil drench before transplanting showed more significant increase in total yield of 124.7; 115.3 and 129.7% for T_1, T_3 and T_2, respectively. Lesser increase in tomato yield was observed in plots treated with mycoherbicides plus foliar application. It is important here to note that glyphosate spraying negatively affected the vegetative growth of tomato plants, so it was important to use spraying commercial fertilizer (Amco-Better, NPK, 20+10+20+TE) to enhance the growth of the plant. This observation was also reported by Vouzounis and Americanos (1998) who found that glyphosate and sulfosate [glyphosate] applied twice at 30 to 50 g a.s./ha were also very effective against broomrape, but reduced the yield of tomato.

The obtained results revealed that different treatments with *T. harzianum* and *T. viride*, either individually or in combination with glyphosate as a foliar spray, successfully reduced both infection and intensity of attack with *O. ramosa* on tomato plants. The developed in the present work technique may be applicable as an effective and cost-effective method for branched broomrape management and control.

CONCLUSIONS

Field trials in Egypt revealed that the application of *T. harzianum* (T_1; T_3) and *T. viride* (T_2) by soil drenching was effective in controlling Orobanche infestation and minimizing the number of spikes parasitic on host plants, pea, faba bean and tomato, and thereby increasing the total yield of these crops.

These *Trichoderma* species applied either singly by soil drenching or in combination with foliar spray of glyphosate reduced both infection and intensity of the attack by *Orobanche* spp. Such an integrated approach of using mycoherbicides and chemical herbicide (glyphosate) reported in this review may be an efficient and cost-effective method for management broomrapes in the areas of Egypt where Orobanche is a problem in production of pea, faba bean, tomato and other winter horticulture and field crops.

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POLISH SUMMARY

PERSPEKTYWY ZWALCZANIA ZARAZY GAŁĘZISTEJ MYKOHERBICYDAMI W EGIPCIE

Gatunki zarazy gałęzistej (*Orobanche* spp.) są ważnymi patogenami bobu, pomidorów i innych zimowych upraw w Egipcie. Jej występowanie jest pospolite i stanowi czynnik ograniczający produkcję tych roślin. W wyniku szeroko zakrojonej lustracji egipskich gleb zakażonych naturalnie tymi patogenami określono w warunkach laboratoryjnych i szklarniowych, 42 izolaty grzybów z rodzaju *Alternaria, Fusarium* i *Trichoderma* będące patogenami zarazy gałęzistej. Następnie badano zdolność trzech izolatów określonych jako *T. harzianum* T_1, *T. harzianum* T_3, *T. viride* T_2 do zwalczania w polu *Orobanche* spp. na grochu, bobie i pomidorze. Wyniki badań wykazały, że traktowanie ziemi wymienionymi gatunkami *Trichoderma* użytymi indywidualnie lub w połączeniu z opryskiwaniem herbicydem glyphosate (50 ppm) było efektywne w ograniczaniu porażenia upraw zarazą gałęzistą oraz powodowało wzrost plonu grochu, bobu i pomidora. Omówiono perspektywy opracowania mykoherbicydów do zwalczania zarazy gałęzistej w Egipcie przy wykorzystaniu *T. harzianum* T_1, *T. harzianum* T_3, *T. viride* T_2 na wyżej wymienionych roślinach uprawnych.