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Applications of plant growth promoting bacteria and *Trichoderma* spp. for controlling *Orobanche crenata* in faba bean

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Abstract

Background: *Orobanche crenata* is an obligate root parasite belonging to Orbanchaceae. Broomrape causes great damage to the faba bean. Several attempts were applied for controlling parasitic weeds. So, the aim of this work is to study the application of *Trichoderma* spp. as well as three rhizobacteria species in comparison to herbicidal effect of Glyphosate (Glialka 48% WSC) for controlling broomrape infesting faba bean (*Vicia faba*).

Materials and methods: Three pot experiments were carried out in the greenhouse of the National Research Centre, Dokki, Giza, Egypt during two successive winter seasons. *Trichoderma* inocula were adjusted to 3.6×10^8 propagules/ml and the bacterium inocula were adjusted at 10^7–10^9 colony-forming unit (CFU)/ml. All treatments were applied, before 1 week of sowing, at rate of 50 ml per pot in experiments I and II, while 100 ml per pot in experiment III.

Results: *Trichoderma* spp. (*T. harzianum*, *T. viride* and *T. vierns*) as well as three rhizobacteria species (*Pseudomonas fluorescens*, *Bacillus subtilis* and *Bacillus pumilus*) enhanced the growth parameters in faba bean plants, i.e. shoot length, shoot fresh weight, shoot dry weight and leaf number in the first experiment when applied without *O. crenata* infection. In the second experiment, all bio-control could protect plants against *O. crenata* infection, where it had better juvenile number reduction, than glyphosate after 2 months of application. Both *B. subtilis* and *B. pumilus* had the highest reduction to juvenile fresh weight, while their effect was equal to herbicide for juvenile dry weight, respectively. The bio-control agents had high effects until the 4th month, but it was less than that of the herbicide. In experiment III, the bio-control agents could highly reduce the juvenile parameters after 2 months, as well as juvenile fresh weight and juvenile dry weight after 4 months, than the herbicide, respectively. The bio-control agents were effective until 6 months, but less than the herbicide effect. All bio-control treatments highly increased the plant growth parameters, than the herbicide.

Conclusion: The application of *Trichoderma* spp. as well as rhizobacteria species could play an important role in controlling broomrape in faba bean as a natural bioherbicide.

Keywords: Glyphosate, Faba bean, *Orobanche crenata*, Rhizobacteria, *Trichoderma* spp.

Background

Broomrape is a parasitic weed widespread in many parts of the world (Brhane et al. 2016) as well as in Egypt (Abdel-Kader and El-Mougy 2009; Ghannam et al. 2012), where it is a major factor limiting faba bean production (*Vicia faba*). Broomrape attacks a wide range of plant species such as carrot, celery, eggplant, faba bean, potato, peppers, peas and tomato. By chemicals released from host roots, the broomrape seeds could germinate, and their seedling roots attack the host plant roots to obtain water and nutrients and remain underground until flowering. Broomrape seeds can be spread by footwear, livestock, machinery, vehicles and flooding (Punia 2014). No single method was effective for economically managing *Orobanche* spp. Therefore, several alternative methods viz. preventive, physical, chemical, agronomic, biological, crop resistance and integrated management were applied for controlling the *Orobanche*. Therefore, we need a single

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cheap method to be effectively, economically and completely controlling this parasite (Habimana et al. 2014; Punia 2014).

The plant growth-promoting bacteria (PGPB) can play a role in protecting plants against *Orobanche* infection. *Pseudomonas fluorescens* showed a high bio-control activity against *Orobanche crenata* and *Orobanche foetida* and positively enhanced the plant growth parameters of faba bean (Zermane et al. 2007). *Pseudomonas marginalis* reduced the incidence of *O. crenata* and improved faba bean (Zermane et al. 2007). *Bacillus circulans*, *Bacillus megatherium* var. *phosphaticum* (BMP) and the combination of BMP plus *Rhizobium leguminosarum* bv. *viceae* significantly reduced the germination of *O. crenata* and increased the dry weight of faba bean (Elabaied et al. 2018).

Application of *Trichoderma harzianum* and *Trichoderma viride*, as soil treatment, followed by spray with glyphosate significantly reduced the broomrape infection and increased the yields of peas, faba bean and tomatoes in the fields, compared with each fungus treatment (Abdel-Kader and El-Mougy 2007, 2009).These treatments may be effective, applicable and cost-effective for controlling *Orobanche*. Several fungi also viz. *Chaetomium* sp., *Fusarium oxysoprum*, *Fusarium solani*, *Rhizoctonia solani*, *Sclerotium rolfsii* and *T. harzianum* could attack the living tissues of *Orobanche ramose* segments causing black lesions, soft rots and complete deterioration within 7 days (Nawar and Sahab 2011). Application of *T. viride* and *T. hamatum* reduced the number of *Orobanche* shoots (Nawar and Sahab 2011). *Trichoderma harzanium*, when applied single or in combination with *Rhizobium legimunosarum*, significantly reduced the disease severity of *O. crenata* and improved the seeds and biomass yield of faba bean varieties (Brhane et al. 2016). *F. oxysporum*, as conidial suspension, significantly decreased the germination and tubercles of *Orobanche*, where the microconidia and chlamydospore formulations greatly diminished the emerged shoots and the germinated seeds of *Orobanche* (Alla et al. 2008). Babalola (2010) found that infested tomato roots with *Fusarium compactum* mycelia plus pectinase treatment (20 U ml^{-1}) had over 50% tubercles dead after 1 week of treatment, while mycelia with mycelia plus cellulose treatment (20 U ml^{-1}) had above 60% mortality.

This work aimed to investigate the application of plant growth-promoting bacteria, i.e. *P. fluorescens*, *Bacillus subtilis* and *Bacillus pumilus* and *Trichoderma* spp., i.e. *T. harzianum*, *T. viride* and *T. vierns* for controlling *O. crenata* infestation and their effects on the growth parameters of faba bean under greenhouse conditions.

Materials and methods

Bio-control agents

The plant growth-promoting bacteria, i.e. *P. fluorescens*, *B. subtilis* and *B. pumilus* as well as *T. harzianum*, *T. viride* and *T. vierns* were obtained from Plant Pathology Department, National Research Centre, Egypt.

Herbicide

The herbicide Glyphosate (Glialka 48% WSC) was applied at recommenced dose (225 cm^3 per hectare) as the control.

Preparation of bio-control agent's inocula

For preparation of *Trichoderma* spp. propagules (colony-forming unit, CFU), the pure culture of each *T.harzianum, T. viride* and *T. vierns* fungal species was maintained on potato dextrose agar in Petri plates at 30 ± 2 °C. For mass production, 1-l conical flasks containing 500 ml of malt extract medium (MEM; 30 g malt extract, 5 g peptone in 1000 ml distilled water) were autoclaved. Then, each flask was separately inoculated with 1-cm-diameter disc of each *Trichoderma* sp. The flasks were inoculated at 30 ± 2 °C for 1 week. Then, the *Trichoderma* inocula were adjusted to 3.6×10^8 propagules/ml by using a haemocytometer slide. Then a mixture of MEM and *Trichoderma* propagules was applied (Abd-El-Khair et al. 2010).

For the preparation of rhizobateria, the pure culture of each bacterium was grown in nutrient broth medium at 30 ± 2 °C for 48 h. For mass production, 1-l conical flasks containing 500 ml of nutrient glucose (2%) broth medium (NGBM; 3 g beef extract, 5 g peptone, 20 g glucose, in 1000 ml distilled water and pH at 7.2) were autoclaved. Then, each flask was separately inoculated with 1.0 ml of broth culture of each bacterium. Then, the inoculated flasks were incubated at 30 ± 2 °C for 48 h and the flasks were shaken twice daily. Each bacterium inoculum was adjusted at 10^7–10^9 colony forming unit (CFU)/ml using dilution method. Each inoculum of bacterial species was applied as mixture of bacterial cells and cultural filtrate (El-Nagdi et al. 2019).

Greenhouse experiments

Three experiments were carried out in the greenhouse of Botany Department, National Research Centre, Egypt. The first experiment was conducted to study the ability of tested bio-control agents for improving growth parameters of faba bean, while the second and third ones were conducted to assess the pathogenic effect of tested bio-control agents against *O. crenata*. The soil free of *O. crenata* contamination was applied in the first experiment, while heavily contaminated soil artificially infected with a seeds bank of *O. crenata* (at the rate of 5% *w*/*w*) was applied in the 2nd and 3rd experiments.

The plastic pots (30-cm diameter), containing 5 kg of a sterilized mixture of loamy soil were arranged according to a completely randomized design on a bench under greenhouse conditions. In experiment I, the treatments

were as follows: (1) Untreated control, (2) MEM only, (3) *T. harzianum*, (4) *T. viride*, (5) *T. vierns*, (6) NGBM only, (7) *P. fluorescens*, (8) *B. subtilis* and (9) *B. pumilus*. All treatments were applied at a rate of 50 ml microbial medium per pot before 1 week of sowing and then all pots were watered. In experiments II and III, pots filled with heavily contaminated soil artificially infected with a seeds bank of *O. crenata* (at a rate of 5% *w*/*w*) was applied and the microbial treatments were as follows: (1) *O. crenata* only, (2) *O. crenata* + glyphosate only, (3) *O. crenata* + NGBM only, (4) *O. crenata* + *P. fluorescens*, (5) *O. crenata* + *B. subtilis*, (6) *O. crenata* + *B. pumilus*, (7) *O. crenata* + MEM only, (8) *O. crenata* + *T. harzianum*, (9) *O. crenata* + *T. viride* and (10) *O. crenata* + *T. vierns*. All treatments were applied at a rate of 50 ml of microbial medium per pot in experiments I and II, while at 100 ml of microbial medium per pot in experiment III before 1 week of sowing and then all pots were watered. Eight seeds of faba bean (Giza 3 cv.) were sown at 3 cm from the soil surface of each pot and then the resulted plants thinned to 4 plants per pot (El-Dabaa et al. 2019). Eight pots were used as replicates for each treatment as well as the controls.

The growth parameters of faba bean plants as shoot length, shoot fresh and dry weights and leaf numbers were recorded in the first experiment at 2, 4 and 6 months of application. Effects of tested bio-control agents in reducing numbers, fresh and dry weights and length of the emerged juvenile *O. crenata* were recorded at 2 and 4 months and 2, 4 and 6 months of applications in experiments II and III, respectively. The same growth parameters of faba bean plants were also recorded at the same periods.

Statistical analysis

Data were subjected to analysis of variance using Computer Statistical Package User Manual Version 3.03, Barkley Co., USA, and mean values of each treatment was compared according to Duncan's multiple range test at $P = 0.05$ level of significance (Snedecor and Cochran 1999).

Results

Experiment I

Effects of rhizobacteria and Trichoderma spp on the growth parameters of faba bean plants without O. crenata infection in pots

Effects of *P. flourencens*, *B. subtilis*, *B. pumilus*, *T. harzianum*, *T. viride* and *T. vierns* on the growth parameters of faba bean plants without *O. crenata* infection in pots are listed in Table 1. After 2 months, application of rhizobacteria treatments enhanced the shoot length (SL), fresh shoot weight (FSW), dry shoot weight (DSW) and leaf number (LN) in the ranges of 4–8%, 24–35%, 23–34% and 21–35% comparing with the controls, respectively. Maximum enhancement effects on FSW, DSW and LN were obtained by *B. pumilus*, whereas *B. subtilis* highly increased the SL. *Tichoderma* spp. increased the same growth parameters in the ranges of 6–9%, 16–31%, 10–32% and 16–32%, respectively. The highest increase in the previously mentioned parameters was obtained by *T. vierns* (SL, FSW and DSW) and *T. viride* (LN), respectively. After 4 months, the growth parameters mentioned before increased by rhizobacteria applications in the ranges of 22–27%, 104–131%, 99–124% and 29–52%, respectively. *P. flourencens* highly enhanced SL, FSW and DSW, while *B. pumilus* highly increased LN. *Trichoderma* spp. improved the growth parameters in the ranges of 14–31%, 43–112%, 32–105% and 27–34%, respectively. *T. vierns* highly increased the SL, FSW and DSW, while *T. viride* highly increased LN. After 6 months, rhizobacteria application increased the growth parameters in the ranges of 17–31%, 7–17%, 33–43% and 11–35%, while the increase with *Trichoderma* spp. was in the ranges of 22–28%, 12–53%, 41–104% and 20–64%, respectively. *B. subtilis* highly improved SL, FSW and DSW, while *P. fluorescens* increased LN. On the other hand, *T. vierns*, followed by *T viride* and *T. harzianum* showed the best growth parameters (Table 1).

Experiment II

Effect of rhizobacteria and Trichoderma spp. on Orobanche crenata parameters

The tested rhizobacteria species as well as *Trichoderma* spp. reduced the *O. crenata* juvenile parameters in pots, under artificial inoculation conditions, as shown in Table 2. After 2 months of application, the rhizobacteria reduced the juvenile numbers (JN) and juvenile fresh weight (JFW) and juvenile dry weight (JDW) in the ranges of 64–75%, 22–61% and 29–57%, compared with control corresponded with the values of 57, 41 and 57% with glyphosate treatment, respectively. *B. pumilus* highly reduced JFW and JDW, whereas *P. fluorescens* highly reduced JN. Many of the previously mentioned parameters were reduced by *Tichoderma* spp. in the ranges of 59–82%, 26–37% and 29–43%, compared to 57, 41 and 57 with glyphosate, respectively. *T. vierns* highly reduced the JFW and JDW, while *T. harzanium* highly reduced the JN, respectively. After 4 months, rhizobacteria application reduced many of the parameters mentioned before in the ranges of 43–60%, 40–55%, 41–52% and 38–52% compared to 68, 65, 67 and 49% with glyphosate, respectively. *P. flourecenss* highly reduced JN, JDW and juvenile length (JL), while *B. subtilis* highly reduced the JFW. *Trichoderma* spp. reduced the juvenile parameters in the ranges of 38–51%, 43–60%, 41–59% and 37–49%, compared to 68, 65, 67 and 49% with

Table 1 Effects of plant growth-promoting bacteria and *Trichoderma* species on growth parameters of faba bean without *Orobanche crenata* treatment under greenhouse conditions (Experiment I)

Treatments	Faba bean growth parameters							
	Shoot length		Fresh weight		Dry weight		Leaf no.	
	cm	Incr.%	g/plant	Incr.%	g/plant	Incr.%	No./ plant	Incr.%
Two months								
Untreated plants	32.9^{a*}	–	19.1^{a}	–	3.49^{a}	–	14.0^{b}	–
Nutrient broth only	33.5^{a}	2	21.1^{a}	11	4.06^{a}	16	16.8^{ab}	20
P. fluorescens	34.1^{a}	4	25.1^{a}	31	4.58^{a}	31	18.6^{a}	33
B. subtilis	35.4^{a}	8	23.6^{a}	24	4.30^{a}	23	16.9^{ab}	21
B. pumilus	34.9^{a}	6	25.7^{a}	35	4.66^{a}	34	18.9^{a}	35
Malt extract medium	33.3^{a}	1	20.5^{a}	7	3.74^{a}	7	15.5^{ab}	11
T. harzianum	34.9^{a}	6	22.3^{a}	17	3.85^{a}	10	17.0^{ab}	21
T. viride	35.1^{a}	7	22.2^{a}	16	4.03^{a}	16	18.5^{a}	32
T. vierns	36.0^{a}	9	25.1^{a}	31	4.59^{a}	32	16.3^{ab}	16
Four months								
Untreated plants	47.5^{c}	–	38.9^{c}	–	5.00^{b}	–	32.8^{c}	–
Nutrient broth only	54.0^{bc}	14	47.9^{c}	23	6.00^{b}	20	33.1^{c}	1
P. fluorescens	60.4^{ab}	27	89.5^{a}	131	11.19^{a}	124	45.8^{abc}	40
B. subtilis	57.8^{ab}	22	79.6^{ab}	104	9.94^{a}	99	42.4^{abc}	29
B. pumilus	59.6^{ab}	26	85.3^{a}	119	10.53^{a}	111	49.8^{a}	52
Malt extract medium	55.8^{ab}	18	51.9^{c}	33	6.49^{b}	30	36.6^{bc}	12
T. harzianum	54.3^{abc}	14	55.6^{bc}	43	6.61^{b}	32	42.6^{abc}	30
T. viride	60.5^{ab}	27	80.3^{ab}	106	9.93^{a}	99	44.0^{abc}	34
T. vierns	62.3^{a}	31	82.4^{a}	112	10.31^{a}	106	41.8^{abc}	27
Six months								
Untreated plants	80.8^{e}	–	79.3^{b}	–	12.63^{c}	–	46.8^{c}	–
Nutrient broth medium	84.5^{de}	5	80.4^{b}	1	15.25^{bc}	21	47.1^{c}	1
P. fluorescens	95.5^{abc}	18	86.5^{ab}	9	16.80^{bc}	33	63.0^{b}	35
B. subtilis	105.6^{a}	31	92.5^{ab}	17	18.00^{bc}	43	54.1b^{c}	16
B. pumilus	94.1^{bcd}	17	85.1^{b}	7	17.20^{bc}	36	52.1^{bc}	11
Malt extract medium	89.0^{cde}	10	80.6^{b}	2	14.75^{bc}	17	51.5^{bc}	10
T. harzianum	98.6^{abc}	22	88.5^{ab}	12	17.83^{bc}	41	56.1^{bc}	20
T. viride	102.3^{ab}	27	94.6^{ab}	19	19.70^{b}	56	63.0^{b}	35
T. vierns	103.3^{ab}	28	108.6^{a}	53	25.73^{a}	104	76.8^{a}	64

*In each column (for each month) followed by the same small letter are not significantly different according to Duncan's multiple range test ($p = 0.05$)

glyphosate, respectively. *T. vierns* highly exhibited fresh weight, dry weight and length of juvenile, respectively (Table 2).

Effect on growth parameters of faba bean

Results in Table 3 revealed that *P. flourencens*, *B. subtilis* and *B. pumilus* increased the SL, FSW, DSW and LN of faba bean, under artificial inoculation conditions, in the ranges of 10–25%, 6–31%, 6–31% and 27–46% after 2 months of application, respectively. *B. pumilus* showed the best increase of SL, FSW and DSW, followed by *B. subtilis* and *P. fluorsecens*, while *B. pumilus* highly increased LN, followed by *P. fluorsecens* and *B. subtilis*. Whereas, *T. harzianum*, *T.viride* and *T. vierns* increased the same parameters in the ranges of 13–24%, 13–30%, 5–29% and 13–34%, compared to increase of 15, 64, 26 and 36% with glyphosate, respectively. Data showed that *T. viride* highly increased SL, FSW and DSW, while *T. vierns* highly increased LN. After 4 months of application, the rhizobacteria increased the SL, FSW, DSW and LN in the ranges of 13–24%, 9–15%, 11–15% and 7–38%, compared to 24, 37, 37 and 30% with glyphosate, respectively. Maximum increase in SL character was obtained by *P. fluorescens*, whereas maximum values of

Table 2 Effects of plant growth-promoting bacteria and *Trichoderma* species on *O. crenata* growth parameters in faba bean under greenhouse conditions (Experiment II)

Treatments	Averages of *O. crenata* growth parameters							
	Numbers		Fresh weight		Dry weight		Length	
	No./plant	Red.%	g./plant	Red.%	g./plant	Red.%	cm	Red.%
Two months								
Orobanche alone (*Orob.*)	4.4^{a*}	–	0.54^{a}	–	0.07^{a}	–	ND	ND
Orob. + glyphosate alone	1.9^{bc}	57	0.32^{bc}	41	0.03^{b}	57	ND	ND
Orob. + nutrient broth medium	3.6^{ab}	18	0.53^{a}	2	0.07^{a}	0	ND	ND
Orob. + *P. fluorescens*	1.1^{c}	75	0.42^{ab}	22	0.05^{ab}	29	ND	ND
Orob. + *B. subtilis*	1.6^{c}	64	0.29^{bc}	46	0.03^{b}	57	ND	ND
Orob. + *B. pumilus*	1.6^{c}	64	0.21^{c}	61	0.03^{b}	57	ND	ND
Orob. + malt extract medium	3.9^{a}	11	0.53^{a}	2	0.07^{a}	0	ND	ND
Orob. + *T. harzianum*	0.8^{c}	82	0.39^{ab}	28	0.05^{ab}	29	ND	ND
Orob. + *T. viride*	1.8^{bc}	59	0.40^{ab}	26	0.05^{ab}	29	ND	ND
Orob. + *T. vierns*	1.0c	77	0.34^{bc}	37	0.04^{b}	43	ND	ND
Four months								
Orobanche alone (*Orob.*)	35.4^{a}	–	2.25^{a}	–	0.27^{a}	–	8.88^{a}	–
Orob. + glyphosate alone	12.5^{b}	68	0.78^{c}	65	0.09^{c}	67	4.50^{cd}	49
Orob. + nutrient broth medium	33.0^{a}	7	1.93^{a}	14	0.22^{ab}	19	7.25^{ab}	18
Orob. + *P. fluorescens*	14.3^{b}	60	1.07^{bc}	52	0.13^{c}	52	5.38^{bcd}	39
Orob. + *B. subtilis*	17.8^{b}	50	1.02^{bc}	55	0.13^{c}	52	4.25^{d}	52
Orob. + *B. pumilus*	20.1^{b}	43	1.34^{b}	40	0.16^{bc}	41	5.50^{bcd}	38
Orob. + malt extract medium	34.0^{a}	4	2.00^{a}	11	0.20^{b}	26	7.75^{abc}	13
Orob. + *T. harzianum*	18.4^{b}	48	1.29^{bc}	43	0.15^{bc}	44	5.13^{bcd}	42
Orob. + *T. viride*	22.1^{b}	38	0.90^{bc}	60	0.11^{c}	59	4.56^{cd}	49
Orob. + *T. vierns*	17.3^{b}	51	1.29^{bc}	43	0.16^{bc}	41	5.63^{bcd}	37

*In each column (for each month) followed by the same small letter are not significantly different according to Duncan's multiple range test ($p = 0.05$)

FSW, DSW and LN criteria were obtained by *B. subtilis*. *Trichoderma* spp. also improved the growth parameters in the ranges of 12–38%, 4–75%, 12–75% and 11–26%, compared to 24, 37, 37 and 30% with glyphosate, respectively. Results showed that *T. vierns* highly increased SL, FSW and DSW, while *T. viride* highly increased LN, respectively (Table 3).

Experiment III

Effect of rhizobacteria and Trichoderma spp. on O. crenata parameters

Application of rhizobacteria species and *Trichoderma* spp. tended to produce significant reduction effect on *O. crenata* infestation as shown in Table 4. After 2 months of application, rhizobacteria reduced JN, JFW and JDW in the ranges of 31–54%, 9–50% and 30–60%, compared to values of 15, 4 and 20% with glyphosate, respectively. *B. pumilus* had the best reduction in JN and JFW, whereas *B. subtilis* highly reduced JDW, respectively. *Tichoderma* spp. reduced *O. crenata* juvenile parameters in the ranges of 31–92%, 7–86% and 50–80% compared to 15, 4 and 20% with glyphosate, respectively. *T. viride* highly reduced *O. crenata* juvenile parameters than other *Trichoderma* spp. After 4 months of application, rhizobacteria reduced the juvenile parameters in the ranges of 11–65%, 66–76%, 65–74% and 5–31%, compared to 92, 36, 37 and 43% with glyphosate, respectively. *B. pumilus* highly reduced JFW, JDW and JL, while *B. subtilis* highly reduced JN. The juvenile parameters' reduction were in the ranges of 6–63%, 50–62%, 51–63% and 8–31% with *Trichoderma* spp., compared to 92, 36, 37 and 43% with glyphosate, respectively. *T. vierns* highly reduced the JFW and JDW, while *T. harzianum* highly reduced the JN and JL. After 6 months of application, the tested rhizobacteria reduced the juvenile parameters in the ranges of 23–64%, 42–65%, 25–64% and 12–65%, compared to 78, 72, 69 and 71% with glyphosate, respectively. *P. fluorescens* highly reduced *O. crenata* parameters than other rhizobacteria. *Trichoderma* spp. reduced the juvenile parameters in the ranges of 6–42-66%, 47–57%, 19–46% and 21–22%, compared to 78, 72, 69 and 71% with glyphosate, respectively. *T. vierns* highly reduced the JN and JFW, while *T. harzianum* highly reduced the JDW and JL, respectively (Table 4).

Table 3 Effects of plant growth-promoting bacteria and *Trichoderma* species on growth parameters of faba bean with *O. crenata* infection, under greenhouse conditions (Experiment II)

Treatments	Growth parameters							
	Shoot length		Fresh weight		Dry weight		Leaf no.	
	cm	Incr.%	g/plant	Incr.%	g/plant	Incr.%	No. /plant	Incr.%
Two months								
Orobanche alone (*Orob.*)	32.4d*	–	16.6[a]	–	3.01[a]	–	10.9[e]	–
Glyphosate alone	37.1[abc]	15	20.8[a]	64	3.79[a]	26	14.8[ab]	36
Orob. + nutrient broth medium	33.6[cd]	4	16.9[a]	2	3.09[a]	3	12.1[cde]	11
Orob. + *P. fluorescens*	35.5[bcd]	10	17.6[a]	6	3.19[a]	6	14.1[abcd]	29
Orob. + *B. subtilis*	40.4[a]	25	19.2[a]	16	3.48[a]	16	13.8[abcd]	27
Orob. + *B. pumilus*	40.5[a]	25	21.7[a]	31	3.93[a]	31	15.9[a]	46
Orob. + malt extract medium	34.9[cd]	8	16.8[a]	1	3.08[a]	2	11.9[e]	9
Orob. + *T. harzianum*	40.0[a]	24	18.8[a]	13	3.15[a]	5	12.8[bcde]	17
Orob. + *T. viride*	39.9[ab]	23	21.5[a]	30	3.89[a]	29	12.3[bcde]	13
Orob. + *T. vierns*	36.5[abcd]	13	19.5[a]	18	3.54[a]	18	14.6[abc]	34
Four months								
Orobanche alone (*Orob.*)	39.5[b]	–	27.8[b]	–	3.48[a]	–	23.4[b]	–
Orob. + glyphosate alone	48.9[ab]	24	38.0[ab]	37	4.76[a]	37	30.5[ab]	30
Orob. + nutrient broth medium	41.9[b]	6	28.5[b]	3	3.70[a]	6	23.6[b]	1
Orob. + *P. fluorescens*	48.9[ab]	24	30.9[ab]	11	3.88[a]	12	25.1[b]	7
Orob. + *B. subtilis*	45.6[ab]	15	32.0[ab]	15	4.01[a]	15	32.3[a]	38
Orob. + *B. pumilus*	44.8[ab]	13	30.4[ab]	9	3.83[a]	11	27.3[ab]	17
Orob. + malt extract medium	43.5[b]	10	28.4[b]	3	3.65[a]	5	24.9[b]	6
Orob. + *T. harzianum*	44.1[b]	12	30.8[ab]	11	3.98[a]	14	26.0[ab]	11
Orob. + *T. viride*	46.1ab	17	28.5[b]	4	3.90[a]	12	27.0[ab]	15
Orob. + *T. vierns*	54.4a	38	48.6[a]	75	6.08[a]	75	29.4[ab]	26

*In each column (for each month) followed by the same small letter are not significantly different according to Duncan's multiple range test ($p = 0.05$)

Effect on growth parameters of faba bean

Under artificial inoculation conditions with *O. crenata*, rhizobacteria species and *Trichoderma* spp. increased the growth parameters of faba bean in pots as shown in Table 5. After 2 months of application, rhizobacteria increased the SL, FSW, DSW and LN in the ranges of 22–38%, 7–51%, 11–53% and 1–31%, while the increases were in the ranges of 19–33%, 22–64 and 23–32% and 7–11% with *Tichoderma* spp., compared to 16, 5, 7 and 2% with glyphosate, respectively. Results revealed that *B. pumilus* highly increased the growth parameters, followed by *P. flourencens* and *B. subtilis*. *T. vierns* highly increased the SL, FSW and DSW, while *T. harzianum* highly increased LN. After 4 months of application, the rizhobacteria increased the growth parameters in the ranges of 5–17%, 12–46%, 15–53% and 9–23%, while *Trichoderma* spp. improved it in the ranges of 6–19%, 11–38%, 16–44% and 5–31%, compared to 2, 9, 5 and 6% with glyphosate, respectively. *B. pumilus* had the highest increase of growth parameters. *T. vierns* highly increased growth parameters, followed by *T. harzianium* and *T. viride*, respectively. After 6 months of inoculation, the rhizobacteria increased the growth parameters in the ranges of 13–21%, 36–43%, 13–24% and 14–30%, while the growth parameters were improved in the ranges of 12–25%, 18–42%, 3–23% and 8–10% with *Trichoderma* spp., compared to 5, 23, 6 and 6% with glyphosate, respectively. *B. pumilus* highly improved the SL, FSW and DSW, while *P. fluorescens* increased leaf number. *T. viride* highly increased growth parameters, than other *Trichoderma* spp., respectively (Table 5).

Discussion

Broomrape is a parasitic weed which decreases the production of legumes, especially faba bean (*Vicia faba*; Rubiales et al. 2016). It is a root holoparasitic plant devoid of chlorophyll and entirely depending on the host for nutritional requirements (Habimana et al. 2014). Application of biotic agents can induce systemic resistance in treated plants against many plant diseases (Walters et al. 2013). Therefore, application of chemical control with systemic herbicides (e.g. glyphosate) at low rates as

Table 4 Effects of plant growth-promoting bacteria and *Trichoderma* species on *O. crenata* growth parameters in faba bean under greenhouse conditions (Experiment III)

Treatments	Averages of *orobanche* growth parameters							
	Numbers		Fresh weight		Dry weight		Length	
	No./plant	Red. %	g/plant	Red. %	g/plant	Red. %	cm	Red. %
Two months								
Orobanche alone (*Orob.*)	1.3^{a}	–	0.56^{a}	–	0.10^{a}	–	ND	ND
Orob. + glyphosate alone	1.1^{a}	15	0.54^{ab}	4	0.08^{b}	20	ND	ND
Orob. + *P. fluorescens*	0.9^{a}	31	0.51^{c}	9	0.07^{b}	30	ND	ND
Orob. + *B. subtilis*	0.9^{a}	31	0.33^{d}	41	0.04^{c}	60	ND	ND
Orob. + *B. pumilus*	0.6^{a}	54	0.28^{e}	50	0.05^{c}	50	ND	ND
Orob. + *T. harzianum*	0.3^{a}	77	0.14^{f}	75	0.04^{c}	60	ND	ND
Orob. + *T. viride*	0.1^{a}	92	0.08^{g}	86	0.02^{d}	80	ND	ND
Orob. + *T. vierns*	0.9^{a}	31	0.52^{bc}	7	0.05^{c}	50	ND	ND
Four months								
Orobanche alone (*Orob.*)	14.3^{a}	–	2.09^{a}	–	0.49^{a}	–	2.41^{a}	–
Orob. + glyphosate alone	1.1^{c}	92	1.33^{ab}	36	0.31^{ab}	37	1.38^{b}	43
Orob. + *P. fluorescens*	12.8^{a}	11	0.64^{b}	69	0.15^{b}	69	1.97^{ab}	18
Orob. + *B. subtilis*	5.0^{bc}	65	0.71^{b}	66	0.17^{b}	65	2.28^{ab}	5
Orob. + *B. pumilus*	12.4^{a}	13	0.50^{b}	76	0.13^{b}	74	1.66^{ab}	31
Orob. + *T. harzianum*	5.3^{bc}	63	1.05^{b}	50	0.24^{b}	51	1.66^{ab}	31
Orob. + *T. viride*	13.4^{a}	6	0.84^{b}	60	0.20^{b}	59	2.22^{ab}	8
Orob. + *T. vierns*	8.6^{ab}	40	0.79^{b}	62	0.18^{b}	63	2.19^{a}	9
Six months								
Orobanche alone (*Orob.*)	13.5^{a}	–	2.61^{a}	–	0.52^{a}	–	9.00^{a}	–
Orob. + glyphosate alone	3.0^{c}	78	0.73^{c}	72	0.16^{c}	69	2.60^{c}	71
Orob. + *P. fluorescens*	4.9^{bc}	64	0.92^{bc}	65	0.19^{c}	64	3.18^{c}	65
Orob. + *B. subtilis*	10.4^{ab}	23	1.52^{b}	42	0.30^{bc}	42	6.25^{b}	31
Orob. + *B. pumilus*	7.0^{bc}	48	1.43^{b}	45	0.39^{ab}	25	7.92^{ab}	12
Orob. + *T. harzianum*	7.8^{bc}	42	1.38^{bc}	47	0.28^{bc}	46	7.00^{b}	22
Orob. + *T. viride*	4.6^{bc}	66	1.12^{bc}	57	0.42^{ab}	19	7.10^{b}	21
Orob. + *T. vierns*	6.0bc	56	1.57b	40	0.41ab	21	7.00b	22

*In each column (for each month) followed by the same small letter are not significantly different according to Duncan's multiple range test ($p = 0.05$)

well as application of fumigation, solarization and cultural practices is possible for controlling broomrape infection, where use of economical and environmentally friendly control is very important (Rubiales and Aparicio 2012). In this study, *P. flourencens*, *B. subtilis*, *B. pumilus*, *T. harzianum*, *T. viride* and *T. vierns* were applied in three separate pot experiments for determining their ability to enhance growth parameters of faba bean as well as decreasing *O. crenata* juvenile parameters. Table 2 illustrates the effect of rhizobacteria on *O. crenata*. Results of experiment II revealed that the tested rhizobacteria and Trichoderma could protect faba bean plants for 4 months against *O. crenata* infection, where most plants were dead after that. The rhizobacteria and *Trichoderma* spp. had better reduction of JN than glyphosate after 2 months of application, while both *B. subtilis* and *B. pumilus* had the highest reduction of JFW; their effect was equal to the herbicide for JDW. Effects of bio-control agents were high until the 4th month, but it was less than the herbicide effect. In experiment III, the tested bio-control agents highly reduced the juvenile parameters after 2 months, while it reduced the JFW and JDW after 4 months more than the herbicide, respectively. The tested bio-control agents were effective until 6 months, but their effect was less than that of the herbicide. These results are in agreement with those recorded by Samejima and Sugimoto (2018) who reported that the weeds can be controlled by using resistant or tolerant varieties, microbiological approach, cultural practices, chemical controls and

Table 5 Effects of plant growth-promoting bacteria and *Trichoderma* species on growth parameters of faba bean with *O. crenata* infection, under greenhouse conditions (Experiment III)

Treatments	Growth parameters							
	Shoot length		Fresh weight		Dry weight		Leaf no.	
	cm	Incr.%	g./plant	Incr.%	g./plant	Incr.%	No./plant	Incr.%
Two months								
Orobanche alone (*Orob.*)	33.1^{d}	–	14.6^{c}	–	1.21^{c}	–	13.0^{b}	–
Orob. + glyphosate alone	38.3^{c}	16	15.3^{c}	5	1.30^{c}	7	13.3^{b}	2
Orob. + *P. fluorescens*	44.4^{ab}	34	19.0^{bc}	30	1.61^{abc}	33	14.5^{b}	12
Orob + *B. subtilis*	40.3^{bc}	22	15.6^{c}	7	1.34^{c}	11	13.1^{b}	1
Orob. + *B. pumilus*	45.6^{a}	38	22.1^{ab}	51	1.85^{ab}	53	17.0^{a}	31
Orob. + *T. virde*	44.1^{ab}	33	24.0^{a}	64	1.95^{a}	61	13.9^{b}	7
Orob. + *T. harzianum*	41.5^{abc}	25	17.8^{bc}	22	1.49^{bc}	23	14.0^{b}	8
Orob. + *T. vierns*	39.3^{c}	19	19.3^{bc}	32	1.60^{abc}	32	14.4^{b}	11
Four months								
Orobanche alone (*Orob.*)	49.0^{b}	–	20.9^{b}	–	3.75^{b}	–	21.0^{b}	–
Orob. + glyphosate alone	50.1^{ab}	2	26.0^{ab}	24	3.93^{b}	5	22.3^{ab}	6
Orob. + *P. fluorescens*	57.1^{ab}	17	22.8^{ab}	9	4.30^{ab}	15	24.6^{ab}	17
Orob. + *B. subtilis*	51.3^{ab}	5	23.5^{ab}	12	4.44^{ab}	18	22.8^{ab}	9
Orob. + *B. pumilus*	52.4^{ab}	7	30.5^{a}	46	5.75^{a}	53	25.8^{ab}	23
Orob. + *T. harzianum*	55.4^{ab}	13	25.5^{ab}	22	4.83^{ab}	29	23.9^{ab}	14
Orob. + *T. viride*	51.9^{ab}	6	23.1^{ab}	11	4.36^{ab}	16	22.0^{b}	5
Orob. + *T. vierns*	58.3^{a}	19	28.8^{ab}	38	5.41^{ab}	44	27.5^{a}	31
Six months								
Orobanche alone (*Orob.*)	70.4^{e}	–	21.0^{b}	–	5.08^{b}	–	25.0^{b}	–
Orob. + glyphosate alone	73.9^{de}	5	25.8^{ab}	23	5.38^{ab}	6	26.4^{ab}	6
Orob. + *P. fluorescens*	79.8^{bc}	13	30.0^{a}	43	6.24^{ab}	23	32.5^{a}	30
Orob. + *B. subtilis*	79.5^{bc}	13	28.5^{a}	36	5.73^{ab}	13	28.5^{ab}	14
Orob. + *B. pumilus*	84.8^{ab}	21	30.1^{a}	43	6.28^{a}	24	29.0^{ab}	16
Orob. + *T. harzianum*	81.9^{bc}	16	24.8^{ab}	18	5.39^{ab}	6	27.0^{ab}	8
Orob. + *T. viride*	88.0^{a}	25	29.8^{a}	42	6.23^{a}	23	32.5^{a}	30
Orob. + *T. vierns*	78.6^{cd}	12	25.0^{ab}	21	5.23^{ab}	3	27.5^{ab}	10

*In each column (for each month) followed by the same small letter are not significantly different according to Duncan's multiple range test ($p = 0.05$)

integrated management. Boari and Vurro (2004) revealed that numerous microorganisms potentially useful for bio-controlling of *Orobanche* species were isolated and reported, but none had been subjected to continuous widespread use. Improving soil fertility also by using beneficial microorganisms appeared to decrease *O. crenata* infestation and its suppressiveness effects on host growth (Elabaied et al. 2018). Application of *T. harzianum, T. viride* and *T. hamatum* reduced the number of *Orobanche* shoots in comparison with the control (Nawar and Sahab 2011; Hyder et al. 2017).

The results cleared that *P. flourencens*, *B. subtilis*, *B. pumilus*, *T. harzianum*, *T.viride* and *T. vierns* could improve the growth parameters of faba bean plants, i.e. shoot length, shoot fresh weight, shoot dry weight and leaf number, when applied with or without *Orbanche* spp. The plant growth-promoting rhizobacteria (PGPR) are rhizospheric microbes produce bioactive substances and promote plant growth and/or protect them against pathogens. Root-colonizing bacteria also improved the plant growth through direct stimulation by producing growth regulators or by suppression of pathogens. PGPR have to be highly competitive to successfully colonize the root zone (Raaijimaker et al. 2002). These results are in agreement with those recorded by Abd-El-Khair et al. (2019) who mentioned that *B. pumilus*, followed by *B. subtilis* and *P. fluorescens* had the highest increase in plant growth parameters in cowpea, respectively. Application of *B. subtilis* and *B. pumilus,* alone or in combination, controlled root-knot nematode and significantly

increased the growth parameters of pea plants such as shoot length, shoot fresh and dry weights, leaf numbers and pod fresh and dry weights in pot experiment (El-Nagdi et al. 2018).

The antagonistic potential effects of both *T. harzianum* (Plantgard) and *B. subtilis* (Rhizo-N) controlled the incidence of *Fusarium* root rot disease and increased the growth parameters per plant viz., shoot length, both fresh and dry shoot weights, branch number, root length and both fresh and dry root weights as well as yield parameters as pod number and fresh and dry weights of pod in faba bean plants in field application in organic farming (Abd-El-Khair et al. 2018). The herbicide Clethodim and three *Trichoderma* strains, as lone or in combination application controlled weeds, root-knot nematodes and *Rhizoctonia* root rot in field applications. The plant growth parameters, (i.e. shoot length, shoot fresh and dry weights and branch and leaf numbers) and yield parameters (fresh pod and dry weights, seed number per pod, seed weight and ash pod weight of plant) were greatly improved with combination treatments when compared to each treatment separately (El-Dabba et al. 2019).

Conclusion

- Broomrape is a weed causing great damage to legumes specially faba bean.
- Several methods were applied to control broomrape.
- This study aimed to apply *Trichoderma* spp. as well as three rhizobacteria species in comparison to herbicidal effect of glyphosate for controlling broomrape infesting faba bean.
- Results revealed that the application of *Trichoderma* spp. as well as rhizobacteria species could play an important role in controlling broomrape in faba bean as a natural bioherbicide as well as enhancing the growth parameters of faba bean.

Abbreviations

B. pumilus: *Bacillus pumilus*; *B. subtilis*: *Bacillus subtilis*; DSW: Dry shoot weight; FSW: Fresh shoot weight; JDW: Juvenile dry weight; JFW: Juvenile fresh weight; JN: Juvenile numbers; LN: Leaves number; *O. crenata*: *Orobanche crenata*; *P. fluorescens*: *Pseudomonas fluorescens*; PGPB: Plant growth promoting bacteria; SL: Shoot length; *T. harzianum*: *Trichoderma harzianum*; *T. vierns*: *Trichoderma vierns*; *T. viride*: *Trichoderma viride*

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Authors' contributions

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The authors declare that they have no competing interests.

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