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Effects of N fertilizer and a bioherbicide on Egyptian broomrape (*Orobanchae aegyptiaca*) in a tomato field

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ABSTRACT- This study aimed to investigate the effects of bioherbicide and nitrogen fertilizer on Egyptian broomrape in tomato field. An experiment was conducted in 2014 at research field of Shiraz University. Treatments consisted of nitrate ammonium (0, 100, 200 and 300 kg ha⁻¹) and biofertilizer as the first factor and bioherbicide (seedling root soaked with the bioherbicide), bioherbigation and control (no bioherbicide) as the second factor. Application of 200 kg ha⁻¹ nitrate ammonium caused a decrease in broomrape height and biomass by 18.7 and 33.7 %, respectively. However, it caused an increase in tomato yield by 26.8%. Bioherbigation also caused a significant decrease in broomrape height and dry biomass by 44.5 and 58.6 %, respectively. Our results showed that application of 200 kg ha⁻¹ nitrate ammonium along with bioherbicide can be a promising strategy to decrease the detrimental impact of broomrape and to increase tomato yield.

INTRODUCTION

Egyptian broomrape (*Orobanchae aegyptiaca*) is an obligate parasite plant species which is widespread in Middle East and Mediterranean agriculture, which can cause heavy yield losses to several crops such as tomato (*Solanum lycopersicum*L.) (Parker and Riches, 1993; Hershenhorn *et al.*, 2009). Orobanchae produces thousands of tiny seeds which are easily disseminated and remain viable in the soil for long period of time (Punia, 2014).

Orobanchae is very hard to control in agricultural crops because of its direct connection with host roots and easily seed dispersal, germination and longevity. Several control strategies for Orobanchae have been used such as cultural and mechanical control methods, delayed planting of host crops, soil-fumigation, soil solarization, trap and catch-crops, biological control, herbicides, herbicide-resistant genetically engineered crops, and resistant cultivars (Foy *et al.*, 1989, Parker and Riches, 1993, Dhanapal *et al.*, 1996). However, most of the aforementioned control approaches were inadequate or difficult to apply or highly costly.

Fusarium oxysporum is known as potential mycoherbicide to control Orobanchae (Hassan Nezhad *et al.* 2005). It has been reported that *Fusarium* species were the most important ones associated with diseased broomrapes. *F. oxysporum* is the predominant species which it possesses several benefits such as relative tolerance to environmental stress in the soil and occurring in the area of distribution of Orobanchae that

make them suitable for the bioherbicide approach. Boutiti *et al.* (2008) suggest that isolated *Fusarium* have the potential to be used as biological control agents against *O. crenata* and *O. foetida* on faba bean (*Vicia faba*) in Tunisia.

Studies on the efficiency of *Fusarium* spp. to control Orobanchae in the field are rare. However, the results showed that *Fusarium* spp. alone in most cases do not provide the level of control desired by farmers (Sauerborn *et al.*, 2007). Application of nitrogen fertilizer appears to be candidates for controlling broomrapes. It has been shown that nitrogen in ammonium form could negatively affect broomrape germination (Haidar and Sidahmed, 2000). Thus, this study conducted to evaluate the efficacy of integrating bioherbicide and fertilization to control broomrape in tomato in Fars Province.

MATERIALS AND METHODS

A field experiment was conducted in 2015 at the research field of Agricultural College of Shiraz University, Shiraz, Iran (29.40°N, 53.35°E, elevation=1810 m above sea level). Soil texture was a silty loam with 0.78 % organic C content, 0.07 % total N, 20.5 mg kg⁻¹ phosphorus, 600 mg kg⁻¹ potassium, pH of 7.80, and an electrical conductivity (EC) of 0.70 dS m⁻¹ in the surface horizon (0–20 cm). Treatments

consisted of four levels of ammonium nitrate (0, 100, 200 and 300 kg ha⁻¹) and biofertilizer phosphonitrokarra (Alkan Yavar company, Iran) (350 ml ha⁻¹ used in the third irrigation) as the first factor, and methods of Orocide bioherbicide application (seedling root treated, bioherbigation at the rate of 20 g m⁻² with the second irrigation and control) as the second factor, which arranged in a strip experiment based on randomized complete block design with three replicates.

Seeds of commercial tomato cv. "Early Urbana Y" (Mid late maturity, globe shaped fruit) were hand sown on February 4, 2015 in a nursery and 4-6 leaves plants were transferred to the field. The seedling was transplanted in 4×5 m plots to a depth of 2 cm. Each plot consisted of 6 rows, spaced 80 cm apart. Irrigation intervals were 3 days until 2 weeks after transplanting and as needed thereafter. However, the irrigation intervals were 4-7 days in spring. Since the cropping system in this area is continues tomato cropping, the field was highly infested with orobanche. The plots were kept free from pests, diseases, and other weeds (hand weeding) during the growing seasons.

Tomato shoot and root biomass, weed biomass, plant height and density of broomrape and plant height and number of fruit per plant were measured. Data were subjected to an analysis of variance (ANOVA) and means were compared with a Duncan's multiple range test (p<0.05) using SAS (version 9.4 2016) and MSTAT-C (version 1.42 1990) software.

RESULTS AND DISCUSSION

Effects of Bioherbicide and Fertilizer on Broomrape Growth

Bioherbicide had a significant effect on broomrape density (p<0.01), but fertilizers had no effect on broomrape density (p>0.05). Bioherbigation and seedling root treated with bioherbicide significantly decreased Orobanche density about 25 % and 51 %, respectively compared to control (Table 1). Bioherbigation was more efficient than seedling root treated with bioherbicide to reduce Orobanche density (51 % vs 25 %). It has been reported that soil application of a simple granular formulation of the fungus (*Fusarium oxysporum* f.sp. *orthoceras*) caused a reduction of approximately 80 % in total number of *O. Cumana* (Habimana et al., 2014). Results of laboratory and greenhouse studies also showed that *F. oxysporum* could control *O. cumana* in sunflower (*Helianthus annuus* L.) properly (Habimana et al., 2014). Cohen et al. (2002) in greenhouse experiments found a reduction in *O. aegyptiaca* attached to tomato using host-specific strains of *F. oxysporum* and *F. arthrosporioides*.

Bioherbicide affected plant height of broomrape significantly (p<0.01), however fertilizer had no significant effect on broomrape height (p>0.05). Bioherbigation and seedling root treated with bioherbicide caused a significant reduction in broomrape height (Table 1). However, bioherbigation was able to reduce broomrape height more than seedling

root treated with bioherbicide (44 % vs 23 %). There was no significant bioherbicide × fertilizer interaction (p>0.05).

Broomrape biomass was affected significantly by bioherbicide (p<0.01). Bioherbigation and seedling root treated with bioherbicide significantly decreased Orobanche biomass about 35 % and 58 %, respectively compared to control (Table 1). Bioherbigation and seedling root treated with bioherbicide caused a reduction of 58 % and 35 % in broomrape biomass, respectively. Boari and Vurro (2004) has reported that use of *F. oxysporum* reduced the number of emerged broomrape shoots by about 70% compared to the control. It also reduced the fresh and dry weights of shoots. Hodosy (1981) obtained excellent results with isolates of *Fusarium oxysporum* which they could reduce more than 90% of broomrape, without any damage to the tomato hosts.

Fertilizer had also a significant effect on broomrape biomass (p<0.05). Application of 300 kg ammonium nitrate ha⁻¹ led to a reduction of 49 % in broomrape biomass. Although other fertilization treatments could also decrease broomrape biomass, 300 kg ammonium nitrate was the superior to the other treatments (Table 3). Mesbah et al. (2012) reported that increase in nitrogen fertilizers could decrease the amount of broomrape germination. It has been also reported that as ammonium nitrate and ammonium sulfate rates increased, shoot number and dry weight of branched broomrape decreased (Mariam and Rungsit, 2004). Since high levels of nitrogen fertilizer or chicken manure showed a suppressive effect on broomrape, its control seems to be associated with less fertile soil conditions (Habimana et al., 2014).

Bioherbicide × fertilizer interaction was significant for broomrape biomass (p<0.05). The lowest amount of broomrape biomass was found in bioherbigation plots which received 200 kg ammonium nitrate ha⁻¹ and the highest was found in control plots (Table 3). This showed that application of ammonium nitrate together with bioherbicide can reduce broomrape growth (Table 3).

Effects of Bioherbicide and Fertilizer on Tomato

Bioherbicide had a significant effect on tomato plant height (p<0.05). The highest plant height obtained in bioherbigation. There was no significant difference between root treated bioherbicide and control. Fertilizer had also a significant effect on tomato plant height (p<0.01). Plants received 300 kg ammonium nitrate had the highest height (Table 2). However, bioherbicide × fertilizer interaction was no significant for tomato plant height (p<0.05).

Number of fruit per plant was affected significantly by both bioherbicide (p<0.01) and fertilizer (p<0.01) but the interaction of biofertilizer × fertilizer was not significant (p>0.05). There was no significant difference between root treated and bioherbigation for number of fruit per plant (Table 1).

Application of 200 kg nitrate ammonium ha⁻¹ caused the greatest number of fruit per plant (Table 2).

Fruit yield of tomato was significantly affected by bioherbicide ($p < 0.01$) (Table 1). Fertilizer had also a significant effect on tomato fruit yield ($p < 0.01$) (Table 2). Bioherbicide \times fertilizer interaction was significant for tomato fruit yield ($p < 0.05$). The greatest tomato yield obtained in plots received 200 kg nitrate ammonium and bioherbigation, which was not significantly different from 200 kg nitrate ammonium and bioherbigation treatment (Table 4). Since most of

the damage to host crops occurs while the parasitic weed is still underground, application of soil-borne biocontrol agents like *Fusarium* spp. can improve crop yield by destroying the parasite at its early developmental stages (Habimana et al., 2014). Mariam and Rungisit (2004) also found that application of ammonium nitrate and ammonium sulfate increased the yields of tomato linearly while they had also a decrease effect on broomrape growth. Ozores-Hampton et al. (2012) total marketable fruits yields showed positive response to N rates.

Table 1. Effects of bioherbicide on broomrape and tomato characters

Bioherbicide	Weed density	Weed height (cm)	Weed biomass (g/plant)	Tomato height (cm)	No. fruit/plant in tomato	Tomato fruit yield (Mg/ha)
Control	7.8 ^a	13.49 ^a	6.3 ^a	43 ^b	13.52 ^b	16.47 ^b
Root treated	5.86 ^b	10.36 ^b	4.06 ^b	42.86 ^b	18.82 ^a	20.61 ^a
Bioherbigation	3.8 ^c	7.48 ^c	2.61 ^c	44.8 ^a	17.21 ^a	21.89 ^a

†In each column, means with the same letters aren't significantly different at $\alpha = 0.05$ by Duncan multiple range test

Table 2. Effects of N fertilizers on broomrape and tomato characters

N fertilizer	Weed biomass (g/plant)	Tomato height (cm)	No. fruit/plant in tomato	Tomato fruit yield (Mg/ha)
0 kg NH ₄ NO ₃ ha ⁻¹	5.92 ^{at}	40.74 ^c	13.48 ^c	17.51 ^b
100 kg NH ₄ NO ₃ ha ⁻¹	4.43 ^b	42.14 ^c	14.91 ^{bc}	19.53 ^b
200 kg NH ₄ NO ₃ ha ⁻¹	3.92 ^{bc}	44.98 ^b	19.38 ^a	23.91 ^a
300 kg NH ₄ NO ₃ ha ⁻¹	2.98 ^c	48.01 ^a	17.72 ^{ab}	19.8 ^b
Biofertilizer	4.38 ^b	41.88 ^c	17.09 ^{ab}	17.54 ^b

†In each column, means with the same letters aren't significantly different at $\alpha = 0.05$ by Duncan multiple range test

Table 3. Interaction effects of bioherbicide \times fertilizer on broomrape biomass (g plant⁻¹)

Bioherbicide	Fertilizer				
	0 kg NH ₄ NO ₃ ha ⁻¹	100 kg NH ₄ NO ₃ ha ⁻¹	200 kg NH ₄ NO ₃ ha ⁻¹	300 kg NH ₄ NO ₃ ha ⁻¹	Biofertilizer
Control	9.3 ^{at}	5.48 ^a	6.2 ^a	3.32 ^a	7.2 ^a
Root treated	5.6 ^b	5.08 ^{ab}	3.61 ^b	2.91 ^a	3.13 ^a
Bioherbigation	2.86 ^c	2.72 ^b	1.95 ^b	2.72 ^a	2.82 ^a

†Means with the same letters in each column aren't significantly different at $\alpha = 0.05$ by Duncan multiple range test

Table 4. Interaction effects of bioherbicide \times Fertilizer on tomato yield (Mg ha⁻¹)

Bioherbicide	Fertilizer				
	0 kg ha ⁻¹	100 kg NH ₄ NO ₃ ha ⁻¹	200 kg NH ₄ NO ₃ ha ⁻¹	300 kg NH ₄ NO ₃ ha ⁻¹	Biofertilizer
Control	13.75 ^{bt}	15.88 ^b	18.25 ^b	17.75 ^a	16.75 ^a
Root treated	22.56 ^a	19.21 ^{ab}	25.3 ^a	19.57 ^a	16.42 ^a
Bioherbigation	16.22 ^b	23.5 ^a	28.17 ^a	22.08 ^a	19.47 ^a

†Means with the same letters in each column aren't significantly different at $\alpha = 0.05$ by Duncan multiple range test.

CONCLUSIONS

It has been reported that no single technique is able to provide complete control of *Orobanche*, therefore integrated approaches which combine several techniques seems to be more effective. Our results showed that application of 200 kg ha⁻¹ nitrate ammonium along with bioherbicide can be a promising strategy to decrease the detrimental impact

of broomrape. This combined treatment could also increase tomato yield. Further works should investigate the combined effects of different management strategy such as chemical, physical, cultural and biological to find the most efficient integrated weed management to reduce the detrimental effect of broomrape in southern Fars Province.

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اثرات علف کش زیستی و کود نیتروژن دار بر گل جالیز (*Orobanche aegyptiaca*) در مزارع گوجه فرنگی

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چکیده این مطالعه با هدف بررسی اثر یک علف کش زیستی و کود نیتروژن بر گل جالیز مصری در مزرعه تحقیقاتی گوجه فرنگی (*Solanum lycopersicum* L.) در دانشکده کشاورزی، دانشگاه شیراز، ایران در سال ۱۳۹۳ انجام شد. تیمارها شامل نیترات آمونیوم (۰، ۱۰۰، ۲۰۰ و ۳۰۰ کیلوگرم در هکتار) و کود زیستی فسفونیتروکارا به عنوان فاکتور اول و علف کش زیستی (آغشته کردن ریشه به علف کش زیستی، کاربرد با آب آبیاری و شاهد (بدون علف کش زیستی)) به عنوان عامل دوم بودند. اثر متقابل علف کش زیستی و کود تنها بر زیست توده گل جالیز و عملکرد گوجه فرنگی اثر معنی داری داشت ($p < 0.05$). استفاده از ۲۰۰ کیلوگرم نیترات آمونیوم در هکتار باعث کاهش ارتفاع گل جالیز و زیست توده آن به میزان ۱۸/۷ و ۳۳/۷ درصد گردید. این تیمار باعث افزایش ۲۶/۸ درصدی در عملکرد گوجه فرنگی شد. علف کش زیستی نیز باعث کاهش معنی دار ۴۴/۵ و ۵۸/۶ درصدی به ترتیب در ارتفاع و زیست توده گل جالیز شد. نتایج نشان داد که استفاده از نیترات آمونیوم همراه با علف کش زیستی می تواند یک استراتژی امیدوارکننده برای کاهش اثرات زیان آور گل جالیز باشد.