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EFFECT OF *BACILLUS* SPP. ON SEED GERMINATION OF SELECTED SPECIES OF THE GENUS *CUSCUTA* (CONVOLVULACEAE)

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Abstract. Species of the genus *Cuscuta* are annual angiospermic rootless and leafless (achlorophyllous) parasitic plants. *Bacillus* is an example of PGPR bacteria exhibiting plant growth promoting activity. In this study the effects of bacterial suspension on germination of dodder's seed has been determinated. Seeds of three *Cuscuta* species were collected from field for evaluating effects of three different *Bacillus* on its germination. Results show that seed germination of the *C. monogyna* and *C. campestris* is inhibited by all three bacterial species. Based on Tukey analysis, the highest inhibitory activity on seed germination of *C. monogyna* was shown with *B. pumilus* (68.88%); as well as C. *campestris* with *B. megaterium* (95.76%) and *B. pumilus* (91.53%), whilst seed germination of *C. europaea* was almost identically inhibited by all three bacterial species. This paper reports the variable effects of *Bacillus* species on the seed germination of selected *Cuscuta* species.

Key words: *Cuscuta, Bacillus*, germination, Iran

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Introduction

The genus *Cuscuta* L. (dodder) comprises about 175 species distributed throughout the world (Holm *et al.* 1997). Seedlings of this parasitic plant produce stems that coil around host plants and build haustoria to penetrate the host tissue and connect to the vascular bundles of the host (Christensen *et al.* 2003).

The germination process takes place in April-May or even in later months; given that certain optimal warmth and moisture conditions are fulfilled. It may occur between May and October, therefore covering the entire vegetative period, but only for those seeds in the upper level of the ground for seeds located in a depth of up to 10 cm (Rousselot & FNAMS 1982).

It is well-known that seeds can stay in the soil and keep their germination ability for a duration of up to 10 years (Rousselot & FNAMS 1982), or even longer (up to 40 years) in the case of those seeds that are preserved under low humidity conditions (FNAMS 1995). An important trait ensuring the success of *C. campestris* Yunck. as a crop parasite is seed dormancy (Hutchinson & Ashton 1980). It is parasite of several crops and weeds (Parker & Riches 1993; Holm *et al.* 1997). Its chemical control is difficult, because of lack of selective herbicides (Dawson 1990) and tolerance to broad-spectrum products e.g. glyphosate (Nadler-Hassar & Rubin 2003).

Plant growth promoting rhizobacteria (PGPR) are groups of bacteria that enhance plant's growth and yield, by producing various plant growth promoting substances as well as biofertilizers. Some common examples of PGPR genera exhibiting plant growth promoting activity are: *Pseudomonas, Azospirillum, Azotobacter, Bacillus* etc. It has been observed that rhizobacteria can act against plants by the production of phytotoxic substances like cyanide (Alstrom & Burns 1989), indole–3–acetic acid (Loper & Schroth 1986) and haterumalide A (Gerhardson *et al.* 2001). The use of rhizobacteria appears as a promising alternative, since some of these microorganisms can suppress the growth of specific plant species (Kremer & Souissi 2001; Hoagland 2001). They can be used directly in soil as bioherbicides (Mazzola *et al.* 1995) or to produce active metabolites against weeds.

Table 1. Voucher information for examined *Cuscuta* species.

Species	Collection data
C. campestris Yunk.	Guilan: Between Rasht and Lashte–nesha, 5 m, 6.Jul.2012, Hadizadeh 4877 (Herbarium of the University of Guilan)
C. monogyna Vahl	Fars: Shiraz, 5m, 12.Sep.2012, Jafari 4878 (Herbarium of the University of Guilan)
C. europaea L.	Mazandaran: Javaherdeh, 1800m, 23.Jul.2013, Hadizadeh 4879 (Herbarium of the University of Guilan)

Essential effects of PGPR on germination and seedling growth of crops have been studied by many researchers (Rodelas *et al.* 1999; Egamberdiyeva 2007; Carvalho *et al.* 2007, 2011), while their effects on weed species seed germination has been less studied (Vrbničanin *et al.* 2008 a, b, 2011). The main aim of this study was the determination of the effects of bacterial suspension on germination of *Cuscuta*'s seed. This phenomenon could be applied for non-chemical weed management as bioherbicides.

Material and methods

Seeds of three *Cuscuta* species (*C. europaea* L., *C. monogyna* Vahl and *C. campestris*) were collected from a field for evaluating effects of three different *Bacillus* on seed germination. Voucher specimens are kept at the Herbarium of University of Guilan in Rasht, Iran (Tab. 1). Immediately before germination, seeds were prepared by soaking them in concentrated H_2SO_4 for 30 or 60 min, (Gaertner 1950). Seeds were then rinsed three times with distilled water. Rhizobacteria selected were: *Bacillus licheniformis* (PTCC: 1721), *B. pumilus* (PTCC: 1319) and *B. megaterium* (PTCC: 1017). This species used in the study were purchased from the Iranian Research Organization for Science & Technology. Control consisted of seeds germinating in water. Incubation of weed seeds was done with 24h old inocula with cell concentration of 108 ml^{-1}. Twenty seeds of *Cuscuta* were selected and placed in each Petri dish and treated with solutions containing the abovementioned bacterial inoculum. In control, only water was added. In treatments, 5 ml of solution containing different bacterial media was added. Three dishes were used for each treatment and control. Germination took place in an incubator (Memmert) at 25°C in the dark.

The seeds were considered to be germinating at the moment of radicle emergence. The number of germinated seeds was recorded daily (germination rate), and the final percentage of germination was measured after 9 days.

Germination rate (sum of germination per day) was calculated using the formula described by Maguire (1962): $M=n1/t1+n2/t2+\ldots+nx/tx$, where *n1, n2, ...nx* are the numbers of the germinated seeds at times *t1, t2, ...tx* in days. Each experiment was conducted three times. All data were processed by analysis of variance (ANOVA) and means were separated by least significant differences (LSD, Tukey) test using statistical software SPSS 17.

Results

The results of LSD analysis show that seed germination of the *C. monogyna* and *C. campestris* is inhibited by all three bacterial species and also this analysis provides significant difference between the treatments and control. Seed germination of the *C. europaea* is inhibited only by *B. megaterium* and two other treatments had no effect on seed germination of this species (Tab. 2). The results of Tukey test showed that *C. monogyna* was restraind more with *B. pumilus* (68.88%); and *C. campestris* with *B. megaterium* (95.76%) and *B. pumilus* (91.53%), while inhibitory activities of the three bacterial species are almost identical on *C. europaea* (Tab. 3).

Table 2. Results of one-way ANOVA analysis LSD for germination as a dependent variable: **C** – control; **B.l.** – *B. licheniformis*; **B.m.** – *B. megaterium*; **B.p.** – *B. pumilus*; **T** – treatment.

Species	(I) T	(J) T	Mean difference(I–J)
C. monogyna	C	B.l.	8.52000*
		B.p.	12.39333*
		B.m.	4.33667*
C. campestris	C	B.l.	1.95333*
		B.p.	2.81333*
		B.m.	2.94333*
C. europaea	C	B.l.	0.16667
		B.p.	0.16667
		B.m.	0.72000*

Note: * The mean difference is significant at the 0.05 level.

Discussion

In this study, effects of different *Bacillus* suspension on seed germination of several *Cuscuta* species were evaluated. Many studies have already been carried out about these microorganisms. It has been reported that *Bacillus* spp. can promote germination and growth of different plant species. It was found that *Bacillus* species have variable effects on seed germination. For example, Vrbničanin *et al.* (2011) found that *Bacillus* species have variable effects (stimulative and inhibitory) on the seed germination of *Ambrosia*. As well, Egamberdiyeva (2007) reported positive effects of *Bacillus* on seed germination. It was shown that the presence of *B. pumilus* and *B. licheniformis* in the alder rhizosphere promote its growth (Probanza *et al.* 1996; Gutierrez-Manero *et al.* 2001). Results of this study confirm data of Sarić & Božić (2009) who found *Bacillus* to have inhibitory effect on germination of *C. campestris* and alfalfa, however, the effect of *B. pumilus* and *B. licheniformis* was weak and irregular. It was clearly observed that *B. pumilus* and *B. licheniformis* do not inhibit the germination of *C. europaea* while *B. megaterium* do it.

A number of previous studies have suggested that *B. pumilus* can be used in bioherbicide formulations (Japan Tobacco Inc. 1998). They showed that only strain 55-30 (Carvalho *et al.* 2007) and strain 83-20 (Carvalho *et al.* 2011) created phytotoxic effects in the lettuce seed germination assay. Obviously, the present results showed that *B. pumillus* inhibits seed germination of *C. monogyna* and *C. campestris* but has no effect on seed germination of *C. europaea.*

The results of this study indicated that *B. megaterium* inhibits seed germination of three *Cuscuta* speices. Although *B. megaterium* can present deleterious effects on weed growth (Kim & Kremer 2005), lettuce seed germination was not affected. However, the reduction of both length and mass occurred for wheat coleoptiles in contact with substances produced by *B. megaterium* (Carvalho *et al.* 2007, 2011).

Studies showed different PGPR have diverse (stimulative or inhibitory) effects on germination of plant species, but it may not be possible to extrapolate the results of these *in vitro* studies to soil or rhizosphere conditions. This is due to the influence of different conditions in the soil (pH, microelements, salinity) and on excretion of plant growth-promoting substances by PGPR (Narula & Gupta 1986). Latour *et al.* (1996) demonstrated that rhizospheric bacteria can have either beneficial effect or be deleterious or neutral, depending on the bacterial species and/or the crop.

This study shows that the most effective bacteria can be selected on the basis of their inhibitory effect on seed germination to be used in weed control management.

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Table 3. Results of one-way ANOVA, Tukey test: **C** – control; **B.l.** – *B. licheniformis*; **B.m.** – *B. megaterium*; **B.p.** – *B. pumilus*; **N** – number of repeats; **T** – treatment.

Species	T	N	Subset for alpha = 0.05			
			1	2	3	4
C. monogyna	B.p.	3	5.6067			
	B.l.	3		9.4800		
	B.m.	3			13.6633	
	C	3				18.0000
C. campestris	B.p.	3	0.2600			
	B.l.	3		1.1200		
	B.m.	3	0.1300			
	C	3			3.0733	
C. europaea	B.p.	3	0.5533			
	B.l.	3	0.5533			
	B.m.	3	0.0000			
	C	3	0.7200			

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