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Binucleate *Rhizoctonia repens* Bernard as a Biocontrol Agent against Damping-off Disease of Cucumber Plants

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ABSTRACT

Efficiency of *Rhizoctonia repens* Bernard, previously isolated from *Spiranthes sinensis* (Pers.) Ames , for biological control of 3 virulent fungal isolates belonging to *Fusarium oxysporum*, *Pythium ultimum* and *Rhizoctonia solani* AG-4 was investigated under *in vivo* conditions. The study, including eight treatments; *R. repens*, *F. oxysporum*, *R. repens*+*F. oxysporum*, *P. ultimum*, *R. repens*+*P. ultimum*, *R. solani* AG 4, *R. repens*+*R. solani* AG 4 and control, was performed in 5 replicates. *R. solani* AG 4 was the most virulent pathogen as compared to the other pathogens. *R. repens* didn't cause any symptoms on cucumber plants in the experiments, and reduced the severity of root rot disease caused by *R. solani*, *P. ultimum* and *F. oxysporum*. There were significant differences between the root length and fresh weight of the plants in pots inoculated with the pathogens alone and that of the plants in pots inoculated with the pathogens plus *R. repens* ($P < 0.05$).

Keywords: *Cucumis sativus* L., biocontrol, binucleate *Rhizoctonia*, soil-borne pathogens

INTRODUCTION

Vegetables are cultivated well in tropical and subtropical regions and also grown in greenhouses or under plastic structure in cooler areas. Different vegetable species were commonly produced in Samsun province, located in the Black Sea Region, with a total greenhouse area of 26.253 decare and production of 144.794 tons/year. Cucumber (*Cucumis sativus* L.), is one of the most important vegateble species produced in the greenhouses in Samsun (Anonymous 2012).

Like other agricultural crops, significant yield losses are caused by soil-borne fungi on cucumbers growing in greenhouses every year (Villajuan-Abgona *et al*. 1996). Root rot and damping-off diseases caused by *Rhizoctonia solani* Kuhn, *Fusarium oxysporum* Schlecht. emend. Snyd. Hans., and *Pythium ultimum* Trow are the most important and common diseases of young cucumber plants (Mitidieri and De Mitidieri 1994; Erper and Karaca 1998).

Control of these pathogens is rather difficult due to their ecological behavior, extremely broad host range and high survival rate of resistant forms such as sclerotia and chlamydospores under different environmental conditions (Yangui *et al*. 2008). Although the use of wide spectrum chemicals is the most efficient and economical control method in small areas like greenhouses, these chemicals effect beneficial microorganisms as well and destroy biological balance in soil (Lifshitz *et al*. 1985). Therefore, studies on biological control, which only decrease populations of pathogens and keep biological balance, gained importance in recent years. For this purpose, many soil microorganisms were investigated and some of them were prepared for practical use (Koch 1999). Many researchers evaluated the potential use of *Rhizoctonia* group fungi against soil borne pathogens as biological control agents. Some multinucleate (MN) *R. solani* and binucleate (BN) *Rhizoctonia* isolates were found to be non-pathogenic and used against pathogenic *Rhizoctonia* spp. (Ichielevich-Auster *et al*. 1985). Additionally, it was also reported that BN *Rhizoctonia* effectively controlled damping off diseases caused by *R. solani*, *Pythium* spp., *Phytophthora* spp., and *Fusarium* spp. in multiple host plants (Cardoso and Echandi 1987; Harris *et al.* 1993; Sumner and Bell 1994; Villajuan-Abgona *et al*. 1996; Cassiolato *et al*. 1997; Burns and Benson 1998; Cartwright and Spurr 1998; Poromarto *et al*. 1998; Xue *et al*. 1998; Muslim *et al*. 2003). BN *Rhizoctonia* were known to have different modes of action in the protection of plants against pathogens. They can form dense mycelial mats on underground plant parts and may compete for nutrients by occupying infection sites (Sneh *et al*. 1989). There is also evidence that they can induce resistance mechanisms of plants (Sneh and Ichielevich-Auster 1995; Xue *et al*. 1998; Hwang and Benson 2003).

Strains of *Ceratobasidium* spp. commonly exist in mycorrhizal associations with orchids (Sneh 1996). These orchid associated *Rhizoctonia* strains are reported to form typical morphological structures in host tissues that were mentioned as monilioid cells (Zelmer *et al.* 1996; Sharma *et al.* 2003). Some BN *Rhizoctonia* species are known to have mycorrhizal associations with orchids and non-pathogenic on other plants. *Rhizoctonia repens* Bernard is one of the first three species reported as orchid endophytes. It was also reported that *R. repens* protected orchid bulbs against pathogens by inducing inhibitory chemicals (Stoessl 1983). Virulence of *R. repens* isolates were investigated on different plants and it was found that the fungus was non-pathogenic on most of the plants and also increased the root lengths of some of them (Ozkoc *et al*. 2002). Similarly, Villajuan-Abgona *et al.* (1996) determined the positive effect of BN *Rhizoctonia* strain W7 on plant growth, as a significant increase in plant fresh weight and height.

Different researchers suggested the timing of the biocontrol inoculation as an important parameter. In a previous study, biocontrol effect of BN *Rhizoctonia* againts fungal pathogens was investigated by inoculating the fungus before or at the same time with the pathogens. Protective

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Short Communication

effects of BN *Rhizoctonia* against *R. solani* were obtained following its pre-inoculation either before short periods like 24 h (Poromarto *et al.* 1998), 48 h (Xue *et al.* 1998) or 7 or more days (Hwang and Benson 2003) before *R. solani* attack.

The objectives of this study were to evaluate the possible use of BN *R. repens* 624 isolate in the biological control of *F. oxysporum*, *P. ultimum* and *R. solani* AG 4 which cause root rot and damping off diseases on cucumber plants, *in vivo*.

MATERIALS AND METHODS

Source of isolates

Three virulent isolates of *Fusarium oxysporum* (isolate 55-05), *Pythium ultimum* (isolate 55-22) and *Rhizoctonia solani* AG-4 (isolate 55-78) were obtained from the cucumber (*Cucumis sativus* L.) plants showing root rot symptoms grown in the greenhouses in Samsun province and that were determined to be virulent on cucumber plants *in vivo*. BN *Rhizoctonia repens* Bernard isolate 624 was obtained from *Spiranthes sinensis* (Pers.) Ames and known to be avirulent on cucumber plants (Ozkoc *et al*. 2002). The cultures of the fungi were maintained on 3.9% potato dextrose agar (PDA, Oxoid Ltd., Basingstoke, Hampshire, England). PDA slants were stored at +4°C and served as stock cultures for further use.

In vivo treatments

The effect of BN *R. repens* isolate on *F. oxysporum*, *P. ultimum* and *R. solani* AG-4 were investigated by *in vivo* experiments. Seeds of MAY Beith Alpha F1 cucumber cultivar were used in the experiment. Cucumber seeds were first surface-disinfected in 70% ethyl alcohol, and then in 1% NaOCl for 5 min. (Ichielevich-Auster *et al*. 1985). After rinsing three times with sterile distilled water, they were blotted dry between sterile paper towels. Five seeds each were planted in plastic pots (1 kg) containing potting mix (field soil, composted manure, sand at 1:1:1, v/v) which was autoclaved for 1 h at 121°C, on 2 successive days. *R. repens* 624 was incubated on PDA at 26 ± 2°C in the dark. In the experiment with *R. repens*, intact agar blocks with *R. repens* grown on PDA (20 ml/plate) were placed into a depth of 1.5-2 cm in a pot, the seeds were sown on the agar blocks and covered with the potting mix. The pots were watered with sterile distilled water and kept in greenhouse conditions with temperatures at 23-30°C. After ten days, 5 agar discs of 5 mm diameter from the growing edges of the cultures of *F. oxysporum*, *P. ultimum* and *R. solani* AG 4 isolates were placed nearby (about 0.5-1 cm) the roots of the plants in the pots. Agar discs without any organism were used as controls. After incubation in the greenhouse for 4 weeks, plants were uprooted and washed. Disease severity was rated by using 1-5 scale modified after Muyolo *et al*. (1993), where 1 = healthy seedling, 2 = very little superficial lesions on roots and hypocothyls, 3 = deep and large lesions on the roots or on the hypocotyls, 4 = severe root rot, lesions surrounding hypocotyl, partially restricted root length, and 5 = complete root-rot. In addition, root lengths and fresh weights of the plants were measured (Ozkoc *et al.* 2002). In the experiment, eight different treatments (*R. repens*, *F. oxysporum*, *R. repens*+*F. oxysporum*, *P. ultimum*, *R. repens*+*P. ultimum*, *R. solani* AG 4, *R. repens*+*R. solani* AG 4 and control) and five replicates were used.

Statistical analysis

All experiments were conducted using completely randomized block design. The fresh weight, root length and disease severity scores were recorded for each plant. The average of the scores of the five plants in each pot represented one experimental unit. The data obtained from the study were subjected to analysis of variance (One Way ANOVA) using the IBM SPSS Statistics Program (version 19, Property of SPSS, Inc., IBM Company, USA), and significant differences between means were determined using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Cucumber cultivation in the greenhouses is gradually growing in Samsun province, Turkey. In connection with the cultivation, root-rot diseases have also been increasing every year in the greenhouses. However, there is not enough effort on the development of effective and safe control strategies especially against diseases caused by *R. solani*, *P. ultimum* and *F. oxysporum*. Chemical control is not satisfactory, and biological control has shown potential as an alternative disease management strategy (Lemanceau and Alabouvette 1993; Larkin and Fravel 1998). Non-pathogenic BN *Rhizoctonia* were found to be an effective biological control agent against such diseases (Burns and Benson 1998; Harris *et al*. 1993; Sumner and Bell 1994; Villajuan-Abgona *et al.* 1996; Cassiolato *et al*. 1997; Cartwright and Spurr 1998; Poromarto *et al*. 1998; Xue *et al*. 1998; Muslim *et al*. 2003). Researchers determined that BN *Rhizoctonia* had several mode of actions that support biological control against plant diseases. These include: (i) forming dense mycelial mats on plant roots, (ii) competing for nutrients by occupying infection sites, (iii) enhancing the resistance mechanisms in BN *Rhizoctonia*-preinoculated plants against pathogens (Sneh *et al.* 1989; Sneh and Ichielevich-Auster 1995; Xue *et al.* 1998; Hwang and Benson 2003).

There is little knowledge about the pathogenicity of BN *R. repens*, known to be mycorrhizal with orchids, on different plants. The results of the present study showed that BN *R. repens* did not cause any symptoms on the cucumber plants. In the statistical analysis, there was also not a significant difference between the growth of cucumber plants in the pots treated only with BN *R. repens* and in the non-treated control pots ($P < 0.05$) (**Table 1**). In the study, *R. solani* was the most virulent pathogen on cucumber plants, followed by *P. ultimum* and *F. oxysporum*, respectively. BN *R. repens* somewhat reduced the severity of root rot disease caused by the pathogens. The antagonistic effect of BN *R. repens* against the pathogens was clear and cucumber plants in pots inoculated with the pathogen plus BN *R. repens* had lower severity than that of the pathogen alone. Our findings were supported by the previous studies having similar results on the use of BN *Rhizoctonia* in the control of diseases caused by *R. solani*, *Pythium* spp., *Phytophthora* spp. and *Fusarium* spp. (Cardoso and Echandi 1987; Cubeta and Echandi 1991; Cartwright and Spurr 1998; Poromarto *et al*. 1998; Xue *et al*. 1998; Burns and Benson 1998; Ozkoc *et al*. 2002).

In this study, fresh weights of the plants in pots inoculated only with BN *R. repens* were higher than that of all the plants in other treatments, but this difference was not statistically significant (**Table 1**). Similarly, the root lengths of the plants inoculated only with *R. repens* was higher than that of control plants, but this difference was not statistically significant. The root lengths of the plants inoculated only with the pathogens were lower than those inoculated with the pathogens plus *R. repens*, except *P. ultimum.* Previous studies reported that BN *Rhizoctonia* promoted plant growth and controlled damping-off disease (Harris *et al*. 1994; Villajuan-Abgona *et al*. 1996; Harris 1999; Ozkoc *et al*. 2002; Chang and Chou 2007). Villajuan-Abgona *et al.* (1996) reported that BN *Rhizoctonia* strain W7 increased plant height and fresh weight. Similarly, Harris (1999) presented results where BN *Rhizoctonia* isolates increased *Capsicum* shoot and root dry weights. Chang and Chou (2007) showed that both orchid mycorrhizal fungi, BN *Rhizoctonia* and *Rhizoctonia solani* AG-6, stimulated the growth of orchid *Anoectochilus formosanus* as compared to non-mycorrhizal plants. It was determined that root length, fresh weight, plant height and number of leaves were enhanced by both fungi.

In many studies, the timing of the biocontrol inoculation was mentioned as an important parameter. Effect of BN *Rhizoctonia* on the biocontrol of fungal pathogens was investigated by inoculating the fungus before or at the same time with pathogens. It was determined that protective

Table 1 Effects of *Rhizoctonia repens* on the disease severity caused by *Fusarium oxysporum*, *Pythium ultimum* and *Rhizoctonia solani* AG 4 and on the root length and fresh weights of cucumber seedlings.

Treatments	Disease severity	Root length (cm)	Fresh weight (g)
R. repens	1.00* ± 0.00 g**	11.52 ± 2.97 a	3.66 ± 0.54 a
F. oxysporum	2.04 ± 0.61 e	7.28 ± 0.96 c	2.60 ± 0.38 bc
R. repens+ *F. oxysporum*	1.84 ± 0.55 f	9.64 ± 0.97 b	2.85 ± 0.36 abc
P. ultimum	3.36 ± 0.91 c	7.34 ± 1.03 c	2.04 ± 0.67 cd
R. repens+ *P. ultimum*	2.80 ± 0.58 d	8.12 ± 0.90 c	2.60 ± 0.45 bc
R. solani AG-4	4.84 ± 0.37 a	0.80 ± 1.64 e	0.37 ± 0.42 e
R. repens+ *R. solani* AG-4	4.00 ± 0.65 b	4.78 ± 1.15 d	1.54 ± 0.53 d
Control	1.04 ± 0.20 g	11.40 ± 1.60 a	3.43 ± 0.39 ab

* Disease severity were evaluated by using 1-5 scale: 1= healthy seedling, 2= very little superficial lesions on roots and hypocotyls, 3= deep and large lesions on the roots or on the hypocotyls, 4= severe root rot, lesions surrounding hypocotyls, partially restricted root length, and 5= complete root-rot.

** Means in a column followed by the same letter are not significantly different from each other according to Duncan's multiple range test ($P < 0.05$).

effect of BN *Rhizoctonia* against *R. solani* were obtained following its pre-inoculation, either short periods like 24 h (Poromarto *et al*. 1998) and 48 h (Xue *et al*. 1998) or 7 or more days before *R. solani* attack (Hwang and Benson 2003). Villajuan-Abgona *et al.* (1996) used three BN *Rhizoctonia* isolates against *R. solani* AG-2-2 and AG-4 causing disease on cucumber plants. They inoculated pathogen isolates 0, 1, 2, 4 and 7 days after BN *Rhizoctonia* inoculation and determined that the protective effect of BN *Rhizoctonia* increased with the increase of interaction periods. Dolan *et al*. (1986) inoculated a BN *Rhizoctonia* isolate 48 hours before *Phytophthora cinnamomi* and *P. citricola* inoculations causing the stem lesions on *Persea* spp., and found that BN *Rhizoctonia* decreased the lesion width. Sneh (1996) reported that BN *Rhizoctonia* were not effective when applied at the same time with the pathogen and mentioned that this was due to the slow growing of BN *Rhizoctonia*. In contrast, there are some reports indicating that BN *Rhizoctonia* were effective on pathogens even if they were inoculated at the same time. Cardoso and Echandi (1987) tested a BN *Rhizoctonia* isolate against MN *Rhizoctonia* causing root rot on bean by inoculating the pathogen and BN *Rhizoctonia* at the same time and determined that the application had protective effect against the pathogen. Similar findings were obtained by Harris *et al*. (1994) as well. In the present study, BN *R. repens* was applied adjacent to the roots of the plants 10 days before the inoculation of the fungal pathogens to the same roots, in order to give BN *R. repens* chance to colonize on plant roots before the pathogen. As a result, BN *R. repens* enhanced plant growth and reduced root rot disease severity caused by the pathogens.

CONCLUSIONS

The results of this study showed that BN *R. repens* increased fresh weight and root lenght of the cucumber plants and reduced the severity of root rot disease caused by *R. solani*, *P. ultimum* and *F. oxysporum* ($P < 0.05$). Since there is not enough knowledge about the pathogenicity of BN *R. repens* on different plants, detailed research is needed to determine its effects on pathogens causing diseases on different plants. Other BN *Rhizoctonia* isolates should also be evaluted for their possible use against plant pathogens.

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