Plant Pathol. J. 26(4) : 346-352 (2010)
DOI: 10.5423/PPJ.2010.26.4.346

The Plant Pathology Journal

Biocontrol of Potato White Mold Using *Coniothyrium minitans* and Resistance of Potato Cultivars to *Sclerotinia sclerotiorum*

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(Received on September 24, 2010; Accepted on November 4, 2010)

This study was conducted in Bahar and Lalehjin, Hamadan, Iran to assess the biocontrol efficacy of *Coniothyrium minitans* Campbell against potato white mold caused by *Sclerotinia sclerotiorum* (Lib.) de Bary under field and greenhouse conditions. In addition, the resistance of common potato cultivars against *S. sclerotiorum* was determined in a greenhouse experiment. After straw inoculation of six potato cultivars (Pashandi, Istambouli, Agria, Marfauna, Alpha and Spartaan) with *S. sclerotiorum*, the least disease severity was observed in Spartaan and Marfauna. Agria showed the most susceptibility to *S. sclerotiorum*. Compared with the healthy control, different concentrations of *C. minitans* conidia (10^7, 10^8 and 10^9 conidia/mL) reduced disease severity under greenhouse condition, and a concentration 10^9 was the most effective treatment. During 2008 and 2009, four field trials were conducted to evaluate the efficacy of *C. minitans* in different soil and aerial applications on disease incidence of potato white mold. In 2008, soil application of Contans® WG (a commercial product of *C. minitans*) showed the greatest biocontrol capacity whereas soil application of solid-substrate *C. minitans* was found inferior when compared with other treatments in both Bahar and Lalehjin field sites. In 2009, benomyl application was the most effective treatment in reducing disease incidence in both tested field sites.

Keywords **:** benomyl, Hamadan, potato cultivars, solid substrate

White mold, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, is a serious problem in a large number of sprinkler-irrigated potato (*Solanum tuberosum* L.) fields in Hamadan, Iran (Ojaghian, 2009). The causal agent of this disease is an ascomycetous fungus which attacks over 400 species of broad-leaved agricultural and horticultural crops (Boland and Hall, 1994). The primary infection of potato plants by this pathogen is initiated by airborne ascospores released from apothecia that are produced from sclerotia in soil (Atallah and Johnson, 2004) and secondary spread of this disease is due to direct contact between infected and healthy tissues (Abawi and Grogan, 1979).

In addition to potato, canola (*Brassica napus* L.) is an economically important and highly susceptible crop cultivated in Hamadan province. Control of *S. sclerotiorum* on potato may help prevent this disease from occurrence within canola fields. Because of wide host range of this pathogen (Steadman, 1979), longevity of sclerotia (Gerlagh et al., 1999), possible infections from ascospores originated from neighboring fields (Yang, 1959), the lack of resistant potato cultivars (Atallah and Johnson, 2004) and economic or environmental concerns over fungicide application, numerous studies have been conducted to explore efficient biocontrol methods to manage this disease on different crops.

Among fungal biological control agents (BCAs), *Coniothyrium minitans* Campbell is the most promising agent for control of *S. sclerotiorum* and when applied to soil, it has been shown to effectively control *S. sclerotiorum* on celery, lettuce (Budge and Whipps, 1991) and sunflower (Huang and Hoes 1980; Huang 1980; McLaren et al., 1994). This mycoparasite was first described for biocontrol of *S. sclerotiorum* in California by Campbell (1947) and has been reported from more than 30 countries on all continents except Antarctica (Sandys-Winsch et al., 1993). Application of this BCA to foliage has been shown to prevent ascospore infection and disease development in alfalfa and beans (Gerlagh et al., 2003; Trutmann et al., 1982) and decrease sclerotial production and survival in rotations of several crops (Gerlagh et al., 1999). This fungus can infect and degrade sclerotia in soil and has the potential to control *S. sclerotiorum* by reducing carpogenic germination, and viability of sclerotia (Huang, 1980; Jones and Whipps, 2002; Tribe, 1957; Trutmann et al., 1980; Whipps and Budge, 1990). *C. minitans* has been shown to survive in soil for several years after application (Budge and Whipps, 1991; McQuilken et al., 1995) and is associated with the development of sclerotinia suppressive soils (Huang and Kozub, 1991). It has been reported that only a single conidium of *C. minitans* is needed to infect a

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sclerotium of *S. sclerotiorum* (Gerlagh et al., 2003) in laboratory conditions but the biocontrol efficacy of *C. minitans* on *S. sclerotiorum* is diminished at high pathogen inoculum levels (Budge and Whipps, 1991; Budge et al., 1995). The optimum temperature for spore germination, infection and destruction of *S. sclerotiorum* sclerotia by *C. minitans* is 20°C (Trutmann et al., 1980) and Hamadan is located in a temperate region in the west of Iran. Commercial product of *C. minitans* (Contans® WG) is usually applied against *S. sclerotiorum* in different infected crops including potato fields (Hammond et al., 2008) and the objective of this study was to evaluate the biocontrol potential of Contans and an Iranian isolate of this mycoparasite in different applications and concentrations against this disease under greenhouse and field conditions in Iran. The resistance of common potato cultivars in Iran to *S. sclerotiorum* was also assessed.

Materials and Methods

Origin and maintenance of *S. sclerotiorum* and *C. minitans*. A highly aggressive isolate of *S. sclerotiorum* (SS18) was used in greenhouse experiments. SS18 had already been isolated in a heavily infected field in Bahar, Hamadan. This isolate was routinely cultured on potato dextrose agar (PDA, 39 g/L; Merck, Darmstadt, Germany) and stored at 4(±1) ºC until used.

One isolate of *C. minitans* (CML1) was obtained from the Culture Collection of Plant Pathology Laboratory, Agriculture College, BuAli Sina University, Hamadan, and stored at 4(±1) ºC on PDA until used. The commercial biocontrol product of *C. minitans* (Contans® WG, Prophyta Biologischer Pflanzenschutz GmbH, Malchow/Poel, Germany) was supplied from the Agricultural Research Center, Mazandaran, Iran.

Greenhouse experiments. To assess the foliar performance of a local *C. minitans* applied at different concentrations against *S. sclerotiorum* on potato (cultivar Agria) and to appraise the susceptibility of six potato cultivars (Pashandi, Istambouli, Agria, Marfauna, Alpha and Spartaan) to *S. sclerotiorum*, pot experiments were conducted in a greenhouse located in Agriculture College, Hamadan, in a completely randomized block design with four replications for each treatment. This experiment was carried out three times in three consecutive weeks in May 2007. Three non-dormant medium potatoes were sown in each 30 cm-diameter plastic pot filled with 2 kg field soil obtained from a potato field nearby Agriculture College, Hamadan. The soil had been pasteurized at 75 ± 5°C for 1 h. The pots were topped with 1 cm of vermiculite and watered as needed for 70 days.

A spore suspension of *C. minitans* was prepared by adding 10 mL of sterile distilled water (SDW) to a 30-day culture grown on PDA and rubbing the surface of the colony using a sterilized glass spatula. The suspension was filtered through four layers of sterile cheesecloth for removal of mycelial fragments and the conidial concentrations were determined using a haemocytometer (Yang et al., 2007).

Plants were sprayed with spore suspension of *C. minitans* at different concentrations (10^7, 10^8 and 10^9 conidia/mL) at the rate of 50 ml/pot. As described by Huang et al. (2000), gum arabic (5 g/L, PelGel; Liphatech, Inc., Milwaukee, WI) and Tween 20 (0.2 ml/L, polyoxyethylenesorbitan monolaurate; Fisher Scientific, Fair Lawn, NJ) were mixed with the spore suspensions. Each concentration replicated four times. Control plants were treated with SDW and the same rate of PelGel and Tween 20.

One hour after spraying, 70-day old plants were inoculated with the pathogen using the straw inoculation method (Auclair et al., 2004). One end of a plastic straw (approximately 5 mm in diameter and 2 cm long) was stapled and the other end was used to bore into the leading edge of a growing culture of *S. sclerotiorum*. The plant stems were cut just below a youngest expanded leaf node and the straw bearing the agar plug was inserted into the cut. To make sure that the lesions weren't a result of damage with the inoculation technique, some other control plants were inoculated with the agar plug only. These control plants were not considered for statistical analyses.

The plants were misted at 20-23 for 72 h after inoculation. Three misting nozzles per block maintained continuous stem wetness for three days after inoculation. After 72 h, the plants were misted for 2 h/day and to determine Disease index (D.I.), lesions were evaluated one and two weeks after inoculation on a scale from 1 to 9, where: 1 = no lesion; 2 = lesion just under the straw; 3 = lesion beyond the straw, but no expanding to the first node from the straw; 4 = lesion expanding to the first node from the straw; 5 = between the first and second node from the straw; 6 = at the second node from the straw; 7 = between the second and third node from the straw; 8 = at the third node from the straw; and 9 = beyond the third node from the straw. The lesions showed to be caused by *S. sclerotiorum* by isolating the pathogen from diseased tissues onto PDA plates. This experiment repeated three times. Because there were four replicated pots for each treatment and each pot had three plants, each of the 36 (3 × 3 × 4) plants was eventually analyzed as a separate replicate. Mean D.I. For each treatment was calculated from 36 replicates.

Six potato cultivars listed above were also tested to ascertain their susceptibilities to *S. sclerotiorum* by the straw inoculation method with 36 replications as mentioned

above, and D.I. was determined one and two weeks after inoculation.

Field testing of *C. minitans* against *S. sclerotiorum.* During growing season of 2008 and 2009, field trials were conducted in two naturally infected potato fields located at Bahar (Dinar Abad) and Lalehjin (Taherloo), two townships in Hamadan province. Both fields had been under potato cultivation for four years. In these field trials, cultivar Agria was sown at 4.8 ton/ha at a row spacing of 35-40 cm. This cultivar is commonly cultivated at Hamadan and has shown high susceptibility to the pathogen. No fertilizer was applied in 2008 while nitrogen fertilizer was broadcast in 2009 tests at a rate of 150 kg/ha prior to planting. Treatments were arranged in randomized complete block design and there were three replicated plots for each treatment in both years. Individual plot sizes were 3.5 × 5.5 m in 2008 and 3.5 × 5 m in 2009, and half-meter wide borders were maintained unplanted between each replicate plot. In both years plots were irrigated with a sprinkler system as needed and weeds were removed by hand. In addition, three plots of diseased fields were remained untreated to act as controls.

In order to provide *C. minitans* inoculum for spore suspension, flasks containing 50 ml tap water and 25 g wheat grains (cultivar Navid) were autoclaved (122°C, 15 min), allowed to cool and then inoculated with two agar plugs (5 mm diameter) taken from the margin of an actively-growing PDA culture of *C. minitans*. After incubation in laboratory condition (the temperature 23 ± 2°C and natural-fluorescent light) for 3 weeks, the inoculated wheat grains were aseptically air-dried overnight and stored at 4°C until used. Spore suspension was made by washing (with autoclaved tap water) the spores off the wheat and the resulting suspension was filtered through four layers of cheesecloth for removal of wheat/mycelial fragments. The concentration of conidial suspensions was determined using a haemacytometer. After it was adjusted to the required concentration, this suspension was mixed with PelGel (5 g/liter) and Tween 20 (0.2 ml/liter). In conidial suspension-used trials, control plots received the same rate of PelGel and Tween 20. Conidial suspension of Contans (granular material containing 1×10^9 conidia/g) was prepared by adding dry material of Contans to tap water and stirring for 30 min. The appropriate dilution (10^9 conidia/mL) for application was prepared on the basis of haemacytometer counts (Jones et al., 2003). As described by McLaren et al. (1994), bran inoculum of *C. minitans* was provided to be applied in soil. Dry wheat bran was moistened with water (850 ml/kg of dry bran), placed in aluminum foil containers, autoclaved at 121°C for 30 min each day for two consecutive days and inoculated with a spore suspension from 25-day PDA cultures of *C. minitans*. The inoculated substrate was remained under laboratory condition for 21-30 days, air-dried for 3 days and stored at 5°C until used in the field. For control plots of bran-used treatments, wheat bran without colonization of *C. minitans* was autoclaved and then air-dried.

In all trials, there were seven identical treatments including foliar spraying of three different concentrations of *C. minitans* (10^7, 10^8 and 10^9 conidia/ml) twice at 50% bloom (in early September at two consecutive weeks), soil spraying of Contans (10^8 spores/m^2) twice in four and two months before sowing potatoes, one foliar spraying of benomyl (200 µg/L, Benlate 50% WP; DuPont) at 1.0 kg/ha at 50% bloom, soil application of *C. minitans* (150 g of solid-substrate inoculum/m^2) two months before sowing potatoes, four aerial applications of *C. minitans* (after tillage in late May, before planting in early June, at 5% and 50% blooming). When no plant was present at field, *C. minitans*/Contans was then incorporated into the soil by rotary-plow to the depth of 20 cm in all above mentioned treatments. Three control plots were allocated for each of treatments. The spore suspensions were applied using a garden hand-pump sprayer at a rate of 300 ml/m^2.

During experimentations, the plots were not irrigated for 24 h after each inoculum application. All plants in each plot were rated for incidence of white mold by visual observation of symptoms (Purdy 1979) during harvesting. Disease incidence was defined as the percentage of plants per plot infected by *S. sclerotiorum.*

Statistical analysis. Analysis of variance (ANOVA) (SAS Institute, Cary, NC, USA, version 6.0, 1989) was used to determine the statistical significance of differences among treatments in the greenhouse and field experiments. Data were pooled when they were not significantly different in the F-test in ANOVA. The percentage data of disease incidence was arcsine-transformed prior to analysis, and the means were back transformed to percentage values after analysis. Treatment means in each experiment were separated using Fisher's protected least significant difference at the $P = 0.05$ level.

Results

Greenhouse experiment. The results after one and two weeks showed that treatments of *C. minitans* (CML1) significantly reduced the *S. sclerotiorum* disease index ($P < 0.05$) compared with the untreated control (Table 1). After one week, concentration 10^9 was the most effective treatment followed by concentrations 10^8 and 10^7 which were not statistically different. Two weeks after straw inoculation, the most biocontrol efficacy was observed in

Table 1. Effect of different concentrations of *Coniothyrium minitans* conidial suspension on disease severity of potato white mold using straw inoculation method in greenhouse condition

Spore concentration	Disease Severity (0-10)	
	week 1	week 2
Untreated control	$6.2^{a*} \pm 2.1$	$8.4^{a} \pm 3.1$
10^{7}	$4.3^{b} \pm 1.3$	$6.1^{b} \pm 2.1$
10^{8}	$4.5^{b} \pm 0.9$	$5.7^{bc} \pm 3.1$
10^{9}	$3.2^{c} \pm 1.2$	$5.5^{c} \pm 1.8$

* Each treatment replicated 12 times. Each value is the mean ± S.E. of four replications. Means followed by the same letters within each column are not significantly different at the $P < 0.05$ level of confidence according to Fisher's test.

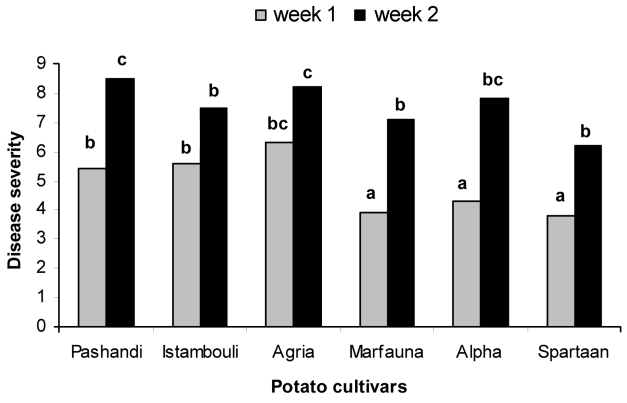

Fig. 1. The comparison of disease severity on six potato cultivars caused by *Sclerotinia sclerotiorum* at one and two weeks of straw inoculation in greenhouse condition. Each treatment replicated 12 times. Values are the means and standard error of twelve replications. Bars having the same letter are not significantly different according to the least significant difference test. Values of bars are significantly different from the healthy control based on orthogonal comparison ($P > 0.05$).

concentration 10^{9} and 10^{8} with no statistical difference between these treatments. Disease index was least inhibited by *C. minitans* applied at a concentration of 10^{7} (Table 1). After One week of straw inoculation on six potato cultivars, Spartaan was shown to be the most resistant cultivar followed by Marfauna and Alpha which were not statistically different. Pashandi and Istambouli were the next most resistant cultivars being not significantly different to each other in DI (Fig. 1). Two weeks after inoculation with *S. sclerotiorum*, DI for Spaartan was significantly lower compared with all other cultivars followed by Marfauna, Istambouli and Alpha with statistically similar performances, and the most susceptible cultivars were Pashandi and Agria with DI of 8.5 and 8.2, respectively (Fig. 1).

Field experiment.

2008 trials: Data from field tests indicated that *C. minitans* treatments can reduce disease incidence of potato white mold (Table 2) and the treatment effects were found significant across both sites and years. At the Bahar field site, soil application of Contans was the most effective treatment ($P < 0.05$) followed by benomyl application with statistically similar results. Next treatment in order of superiority was four foliar applications of *C. minitans* and it was followed by two times of foliar spraying of *C. minitans* (10^{8}) and *C. minitans* (10^{9}) with statistically similar performances. Compared to the control, the least biocontrol efficacy was observed in two times of foliar spraying of *C. minitans* (10^{7}) and soil application of *C. minitans* (Table 2). At the Lalehjin field site, soil spraying of Contans caused maximum biocontrol efficacy ($P < 0.05$) followed by four times of foliar *C. minitans* with statistically similar results. Benomyl, *C. minitans* (10^{7}), *C. minitans* (10^{8}) and *C. minitans* (10^{9}) were the next

Table 2. Efficacy of different treatments of *Coniothyrium minitans* (CM) on disease incidence of potato white mold (field experiments, 2008 and 2009)

Treatments	Disease incidence (%)			
	2008		2009	
	Bahar	Lalehjin	Bahar	Lalehjin
Control	$85^{a*} \pm 28.2$	$74^{a} \pm 34.6$	$79^{a} \pm 29.8$	$69^{a} \pm 29.8$
Twice foliar CM (10^{7})	$46^{b} \pm 21.9$	$25^{c} \pm 12.5$	$31^{b} \pm 15.2$	$31^{c} \pm 15.3$
Twice foliar CM (10^{8})	$37^{c} \pm 14.8$	$29^{cd} \pm 16.5$	$25^{b} \pm 14.2$	$28^{c} \pm 8.5$
Twice foliar CM (10^{9})	$39^{c} \pm 18.2$	$29^{cd} \pm 8.8$	$18^{c} \pm 6.4$	$28^{c} \pm 14.1$
Soil spraying of Contans	$21^{d} \pm 11.2$	$18^{d} \pm 7.7$	$24^{bc} \pm 11.6$	$25^{cd} \pm 11.8$
Four times of aerial CM	27 ± 9.4^{cd}	$20^{d} \pm 8.8$	$18^{c} \pm 5.4$	$15^{d} \pm 3.8$
Soil application of CM	$48^{b} \pm 23.4$	$41^{b} \pm 19.8$	$21^{bc} \pm 14.1$	$45^{b} \pm 11.2$
Benomyl	$25^{d} \pm 9.6$	$24^{c} \pm 15.1$	$12^{d} \pm 8.9$	$10^{d} \pm 2.4$

* Each treatment replicated three times. Means followed by the same letters within each column for each year are not significantly different at the $P < 0.05$ level of confidence according to Fisher's test.

treatments in order of superiority and the least biocontrol efficacy was observed with the soil application of *C. minitans* (Table 2).

2009 trials: Results of both field trials revealed a significant ($P < 0.05$) suppression of sclerotinia white mold by different treatments. In Bahar field, the benomyl treatment significantly reduced disease incidence compared with all other treatments. The next most effective treatments were four times of aerial *C. minitans*, *C. minitans* (10^9), soil application of *C. minitans* and soil spraying of Contans which were not significantly different from each other. *C. minitans* (10^7) and *C. minitans* (10^8) were the least effective treatments with statistically at par results (Table 2). In Lalehjin trial, benomyl and four times of foliar *C. minitans* were the most efficient treatments which were not significantly different to each other. Contans, *C. minitans* at 10^9, *C. minitans* at 10^8 and *C. minitans* at 10^7 were the next most effective treatments being not significantly different from each other. The soil application of *C. minitans* was found inferior than other treatments.

Discussion

Center pivot irrigation is a stimulant factor for sclerotinia diseases (Blad et al., 1978; Hunter et al., 1984), and may be the most important cause for highly occurrence of potato white mold at Hamadan province. To test the non-chemical control of potato white mold, this research was arranged to evaluate the resistance of common potato cultivars, and also test the biocontrol efficacy of *C. minitans* on *S. sclerotiorum* in aerial and soil applications. Because sclerotia are source of primary infection, search for the best BCAs for controlling this pathogen is essential to aim at finding effective microorganisms to destroy sclerotia in soil as well as holding back the symptoms on plant tissues. The results in two years of field trials were relatively consistent and showed that *C. minitans* has potential for biological control of potato white mold.

The results of straw inoculation on six potato varieties demonstrated that all cultivars are susceptible to *S. sclerotiorum*, and this confirms the previous reports (Atallah and Johnson, 2004). Agria and Marfauna are two commonest varieties cultivated at Hamadan and this trial showed that Agria is more susceptible than Marfauna.

Based on results in greenhouse and field tests, aerial application of *C. minitans* is an effective strategy for biocontrol of potato white mold. There are some reports showing that spraying *C. minitans* conidia onto foliage can prevent ascospore infection and disease development in alfalfa, beans and oilseed rape (Huang et al., 2000; Gerlagh et al., 2004; Li et al., 2003; 2005; 2006). The glasshouse experiments also confirm the report of Bremer et al. (2000) indicating that *C. minitans* can directly suppress petal-mediated infection of plant leaves by ascospores of *S. sclerotiorum*. Based on previous researches, Foliage application of *C. minitans* is able to diminish sclerotial production and survival in rotations of several crops (Gerlagh et al., 1999) and spraying on crop debris caused decrease in sclerotial carryover (Budge et al., 1995). The concentration of conidial suspension of *C. minitans* was a significant factor in reducing disease severity in greenhouse and field trials, and this corroborates the results of a biocontrol study on rapeseed (Li et al., 2006) which reported that for the factors tested (*C. minitans* conidial concentration, *C. minitans* isolate and rapeseed cultivar), conidial concentration of *C. minitans* was the most important for biocontrol efficacy. Bremer et al., (2000) emphasized the importance of establishing and maintaining *C. minitans* populations on the phyllosphere and anthoplane of rapeseed to a level that is adequate for suppression of sclerotinia diseases.

Solid-substrate inoculum of *C. minitans* reduced disease incidence significantly but this type of inoculum was observed less efficient than other tested treatments. Many experimental systems have applied solid substrate fermentation products directly to soil with concomitant arguments about cost effectiveness (Whipps and Gerlagh, 1992). Bogdanova et al. (1986) reported that when *C. minitans* was applied to soil in the fall, its hyperparasitic activity was not apparent until spring, when temperature increased. This can explain the relatively low efficacy of the solid substrate wheat inoculum in these field experiments. It is also likely that spraying of *C. minitans* can put spores on the soil surface more evenly and effectively than use of solid substrate. McLaren et al. (1994) found that *C. minitans* grown on bran was more effective than grown on limestone/bran mixture to decrease *S. sclerotiorum* incidence on sunflower, therefore it is necessary to test the efficiency of this biocontrol agent on different substrates in Hamadan fields.

In most of field tests, *C. minitans* spraying onto blooms was less effectual treatment than benomyl application. This finding is consistent with a study in Canada (Huang et al., 2000) where *C. minitans* was less effective in suppressing dry bean (*Phaseolus vulgaris* L.) white mold than benomyl. Moreover, it has been reported that a high incidence of white mold in fungicide treated bean crops is caused by the presence of unprotected flowers under conditions of high inoculum and favorable weather (Hunter et al., 1984; Steadman, 1983).

In this study, Contans was observed as an effective BCA in reducing disease incidence. This commercial biocontrol product is based on *C. minitans* conidia produced by solid-state fermentation and has been shown to suppress sclerotinia disease (Jones et al., 2004). As reported by Partridge et

al. (2004), Contans is able to reduce *Sclerotinia minor* infection of peanut, and in oilseed rape fields infected by *S. sclerotiorum* is a viable alternative to the control by chemical fungicides (Yang et al., 2007). A period of eight weeks has been recommended between Contans application and planting and the recommended application rate (de Vrije et al., 2001) of this product is 10^8 spores/m^2 (10^{12} spores/ha).

As observed in Table 2, disease incidence is different between treatments in two years of this study. The reason for this result might be the environmental factors (temperature and rainfall precipitation) affecting sclerotial survival, sclerotial germination or development of mycelia of *S. sclerotiorum* on the plants (Yang, 1959). Previous studies have showed that the disease pressure may affect the efficacy of biocontrol agents including *C. minitans* (Trutmann et al., 1982; Huang et al., 2000). In addition plants can be infected by ascospores arising from neighboring infected fields/plots (Yang, 1959) which may have contributed to the variation in disease observed in the current study. In this study, *S. sclerotiorum* infected fields were surrounding experimented field sites in the two years.

In New Zealand, different isolates of *C. minitans* showed differences in ability to infect sclerotia of *S. sclerotiorum* (Jones and Stewart, 2000) therefore it is necessary to search for more efficient isolates in Iran. Although CML1 showed good results in this study, efficacy of this isolate can be improved by testing different application strategies, targeting the biocontrol agent more effectively (at the disease debris at the end of the season as well as soil and foliar applications). It is also essential to develop integrated control strategies and consider cultural methods such as drip irrigation systems and increase the row spacing in potato fields in Hamadan. Some experiments are also necessary to show if *C. minitans* is able to overwinter under the weather conditions of Hamadan, and subsequently parasitize sclerotia of *S. sclerotiorum* in the soil.

Acknowledgments

The author thanks Dr. Doostmorad Zafari, Dr. Gholam Khodakaramian and Dr. Mohammad Javad Soleimani for their technical supports and for their advices to improve this article. I gratefully appreciate the various members of Plant Pathology Lab., Agriculture College, Bu Ali Sina University for help with this study.

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