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INHIBITORY EFFECT OF FORMULATED BIOCONTROL AGENTS TOGETHER WITH REDUCED RATES OF IPRODIONE AGAINST POTATO WHITE MOLD

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ABSTRACT: This study was conducted during the cropping seasons of 2016 and 2017 to evaluate inhibitory effect of *Coniothyrium minitans* as well as three isolates of *Trichoderma* viz. *Trichoderma ceramicum*, *T. atroviride* and *T. viridescens* together with reduced rates of iprodione against *Sclerotinia sclerotiorum*. The results showed that in both years, inhibitory effect of each biocontrol agents together with reduced application of iprodione at 80% and 60% the recommended rate was statistically on par with application of the fungicide at 100% the factory advised rate.

KEYWORDS: Bahar; *Coniothyrium minitans*; Hamedan; *Sclerotinia sclerotiorum*; *Trichoderma* spp.

INTRODUCTION

Potato white mold is an economically important disease in many potato growing regions throughout the world. The causal agent, *Sclerotinia sclerotiorum* (Lib.) de Bary, has a broad host range which attacks over 400 dicotyledonous plants (Purdy, 1979). Potato white mold is favored by moist conditions usually occurred in the fields with over head irrigation. This disease has caused substantial yield losses in recent years in Hamedan, Iran (Ojaghian, 2011).

The majority of local farmers have propensity toward applying effective fungicides such as iprodione and dichloran (Ojaghian *et al*., 2015). Although fungicides are able to kill the pathogen, their preventive application is considered as an expensive control method. Failure to determine the right time of fungicide application adds a considerable cost to the farmers. In addition, several studies have shown inefficacy of carbendizem and dimethaclon fungicides against *Sclerotinia* diseases due to development of fungicide resistance (Wang *et al*., 2015; Hu *et al*., 2016). Therefore, it is necessary to introduce sustainable control methods in order to reduce fungicides load in potato fields.

Application of *Trichoderma* spp. and *Talaromyces flavus* into soil have been reported to reduce incidence of white mold (10-50%) significantly in Hamedan potato fields (Ojaghian, 2011) but this level of disease control is not acceptable to potato growers. Hence, new methods are needed to increase inhibitory efficacy of the biocontrol agents. Formulations influences different aspects of the success of biocontrol agents, including the shelf-life of a product, ability of a biocontrol agents to proliferate and survive in the environment, efficacy of disease control, ease of preparation, application and expense (Lumsden *et al*., 1995).

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MATERIALS AND METHODS

In this study, field experiments were conducted to test inhibitory effect of formulated biocontrol agents in combination with lower rates of iprodione against potato white mold. One isolate of *Coniothyrium minitans* (CML1, Ojaghian, 2009) and three isolates of *Trichoderma* including *T. ceramicum* (Tceb1), *T. atroviride* (Tatl 2) and *T. viridescens* (Tvig 2) were kindly provided by culture collection of Agriculture College, Bu Ali Sina University, Hamedan, Iran. The *Trichoderma* isolates were able to infect sclerotia, decompose rice straw, and show high inhibitory effect against *S. sclerotiorum* under *in vitro* and *in vivo* conditions in earlier studies (Ojaghian, 2011). The *Trichoderma* isolates were formulated in 50% oilseed rape seedcake (Guizhou Farmers' Oil House Co., Ltd., Guizhou), 20% rice straw (obtained from a grain harvested field in Gilan Province, Iran), and 30% water (w/w/v) as described by Min (2008) and Hu *et al*. (2015). Maizemeal-perlite inoculum of *C. minitans* was provided by adding 1.5 L of perlite, 400 ml of ground maize and 500 ml of tap water into mushroom spawn bags. After autoclaving, the bags were inoculated with 120 ml of a spore suspension of *C. minitans* which was prepared according to Ojaghian (2010). This inoculum contained between 8×10^8 to 5×10^9 CFU/ cm^3 fresh material.

Field trials were conducted over two years in two naturally highly infested potato fields located at Bahar and Lalehjin, two counties in Hamedan province, during the cropping seasons of 2016 and 2017. The Bahar and Lalehjin fields had been under potato cultivation for five years, and having clay loam (pH 6.1) and sandy clay loam (pH 6.3) soils, respectively. Field tests were arranged in a randomized complete block design with three replications. Tubers (cv. Agria) were planted in a plot size of 4 x 5 m at a row spacing of 35-40 cm (also give plant to plant spacing). One-meter wide borders were maintained between each replicated plot. Fertilizers mainly urea (160 kg/ha), superphosphate (140 kg/ha) and potassium chloride (165 kg/ha) were applied at the time of planting. The treatments included T1: iprodione @ 100% of recommended rate, T2: iprodione @ 80% of recommended rate, T3: iprodione @ 60% of recommended rate, T4: iprodione 40% of recommended rate, T5: *Coniothyrium minitans* @ 200 g/plot, T6: *T. ceramicum* @ 200 g/plot, T7: *T. atroviride* @ 200 g/plot, T8: *T. viridescens* @ 200 g/ plot, T9: consortia of all biocontrol agents (50 g each) @ 200 g/plot, T10-T13: same as treatments T5-T8 except iprodione was also sprayed at 100% the recommended rate, T14-T17: same as treatments T5-T8 except iprodione was also sprayed at 80% the recommended rate, T18-T21: same as T5-T8 except iprodione was also sprayed at 60% the recommended rate, T22-T25: same as treatments T5-T8 except iprodione was also sprayed at 40% the recommended rate, and T26: no treatment. Biocontrol agents were applied into soil at the time of planting. The fungicide iprodione (Rovral WP, 50% a.i.; recommended rate 500 g ha^{-1}, Bayer Ltd., Philadelphia, USA) was sprayed when approximately 65-70% of the potato plants were flowering. In both years plots were irrigated with a sprinkler system as needed and weeds were removed by hand.

Incidence of white mold was recorded at the time of harvesting (late September). Besides, observations were also recorded on percentage of dead plants per plot. Means of treatments recorded in percent were converted in Sin^{-1} percentage transformation according to Gomez and Gomez (1984). Using SAS software (SAS 8.2, 1999–2001; SAS Institute Inc., Cary, NC), the effects of different treatments were evaluated by ANOVA in completely randomized design tests.

RESULTS AND DISCUSSION

The results revealed that, in general, combination of diminished rates of iprodione with formulated biocontrol agents (P>0.05) were as effective as application of iprodione at recommended rate (Table 1). Minimum percent of infected plants in Bahar during 2016 was observed in the treatments T1 and T10-T21 followed by T2 and T3 and these were statistically at par with each other. The treatments T1-T2 and T10-T21 caused minimal infected and dead plants in Lalehjin during 2016 at a statistically similar level. The highest percentage of infected plants in Bahar during 2016 was observed in T23 compared with control. Moreover,

Table 1. Efficacy of formulated biocontrol agents in combination with lower rates of iprodione against white mold

	Disease incidence (%)*							
	2016				2017			
	Bahar		Lalehjin		Bahar		Lalehjin	
Treatments	Infected plants	Dead plants	Infected plants	Dead plants	Infected plants	Dead plants	Infected plants	Dead plants
T1	21.9 ± 7.4^{d}	4.3 ± 2.8^{d}	15.4 ± 5.2^{c}	3.5 ± 0.8^{c}	16.7 ± 5.9^{d}	5.3 ± 2.4^{e}	14.5 ± 3.2^{e}	7.8 ± 1.5^{c}
T2	38.4 ± 10.1^{c}	18.6 ± 5.2^{c}	17.8 ± 11.5^{c}	4.9 ± 1.7^{c}	37.8 ± 4.9^{c}	19.8 ± 1.3^{d}	38.4 ± 11.7^{d}	12.4 ± 0.9^{bc}
T3	41.3 ± 16.5^{c}	16.7 ± 9.6^{c}	56.3 ± 14.1^{b}	23.3 ± 1.1^{b}	39.6 ± 11.6^{c}	21.8 ± 9.7^{d}	36.1 ± 9.2^{d}	14.1 ± 2.2^{bc}
T4	66.4 ± 9.7^{b}	25.2 ± 13.6^{bc}	73.8 ± 7.4^{a}	26.4 ± 5.9^{b}	55.9 ± 20.8^{b}	46.7 ± 15.2^{b}	48.1 ± 12.6^{c}	21.3 ± 4.1^{b}
T5	50.9 ± 12.8^{bc}	32.3 ± 5.9^{b}	57.8 ± 14.2^{b}	22.7 ± 9.2^{b}	40.1 ± 9.7^{c}	36.7 ± 11.3^{c}	59.2 ± 5.8^{b}	25.4 ± 2.6^{b}
T6	63.1 ± 15.7^{b}	41.5 ± 10.2^{ab}	71.1 ± 11.5^{a}	44.6 ± 12.5^{a}	52.4 ± 11.6^{b}	34.8 ± 12.7^{c}	62.5 ± 14.3^{b}	24.8 ± 6.7^{b}
T7	61.2 ± 9.4^{b}	39.8 ± 9.7^{ab}	67.9 ± 12.4^{ab}	42.7 ± 8.3^{a}	54.9 ± 7.3^{b}	37.9 ± 8.2^{c}	63.4 ± 12.2^{b}	25.1 ± 5.4^{b}
T8	65.3 ± 17.5^{b}	40.9 ± 6.4^{ab}	72.6 ± 18.2^{a}	46.8 ± 21.1^{a}	51.5 ± 10.8^{b}	38.6 ± 14.5^{c}	58.1 ± 7.9^{b}	20.1 ± 9.3^{b}
T9	63.9 ± 7.2^{b}	43.3 ± 8.6^{ab}	73.5 ± 14.9^{a}	43.9 ± 17.2^{a}	55.3 ± 14.7^{b}	35.9 ± 7.8^{c}	61.4 ± 20.3^{b}	22.8 ± 1.6^{b}
T10	17.5 ± 3.9^{d}	5.5 ± 1.2^{d}	16.4 ± 4.8^{c}	3.6 ± 0.5^{c}	15.3 ± 5.9^{d}	3.4 ± 0.8^{e}	15.6 ± 4.9^{e}	6.1 ± 0.9^{c}
T11	18.8 ± 5.4^{d}	4.9 ± 1.9^{d}	17.2 ± 8.5^{c}	5.1 ± 1.8^{c}	17.6 ± 3.2^{d}	4.5 ± 1.2^{e}	16.1 ± 5.3^{e}	9.2 ± 3.5^{c}
T12	21.9 ± 4.3^{d}	6.1 ± 2.5^{d}	15.4 ± 5.7^{c}	4.6 ± 1.3^{c}	14.8 ± 6.5^{d}	3.5 ± 0.2^{e}	14.1 ± 3.2^{e}	7.1 ± 1.4^{c}
T13	20.1 ± 5.7^{d}	3.8 ± 1.1^{d}	17.3 ± 6.3^{c}	3.9 ± 0.7^{c}	15.7 ± 1.6^{d}	2.3 ± 0.6^{e}	17.5 ± 6.4^{e}	8.7 ± 2.5^{c}
T14	17.5 ± 8.3^{d}	6.8 ± 2.3^{d}	18.2 ± 9.5^{c}	4.9 ± 2.1^{c}	13.9 ± 5.8^{d}	5.1 ± 1.7^{e}	16.5 ± 3.7^{e}	6.3 ± 0.7^{c}
T15	18.7 ± 4.9^{d}	5.5 ± 1.8^{d}	16.6 ± 5.3^{c}	5.3 ± 1.6^{c}	15.1 ± 4.7^{d}	4.8 ± 1.2^{e}	15.9 ± 4.6^{e}	7.8 ± 1.2^{c}
T16	16.9 ± 7.1^{d}	4.5 ± 1.6^{d}	20.4 ± 8.1^{c}	4.2 ± 1.8^{c}	15.8 ± 7.2^{d}	5.2 ± 1.5^{e}	16.1 ± 5.5^{e}	8.9 ± 2.4^{c}
T17	18.3 ± 9.4^{d}	6.2 ± 2.4^{d}	18.3 ± 10.5^{c}	5.5 ± 1.9^{c}	14.3 ± 5.1^{d}	6.1 ± 2.6^{e}	14.8 ± 2.7^{e}	9.1 ± 3.3^{c}
T18	23.4 ± 2.5^{d}	7.1 ± 2.8^{d}	17.3 ± 3.6^{c}	5.9 ± 2.6^{c}	16.1 ± 7.2^{d}	4.9 ± 1.8^{e}	17.1 ± 6.3^{e}	7.2 ± 0.5^{c}
T19	22.9 ± 4.8^{d}	5.3 ± 1.6^{d}	19.8 ± 8.7^{c}	6.2 ± 1.8^{c}	15.9 ± 4.3^{d}	5.5 ± 0.8^{e}	15.1 ± 4.7^{e}	8.5 ± 2.4^{c}
T20	24.1 ± 7.3^{d}	5.9 ± 2.2^{d}	18.2 ± 6.5^{c}	5.5 ± 1.3^{c}	14.8 ± 2.5^{d}	4.6 ± 1.9^{e}	14.8 ± 5.5^{e}	9.2 ± 1.8^{c}
T21	23.7 ± 6.6^{d}	6.8 ± 1.4^{d}	20.4 ± 5.2^{c}	4.9 ± 1.1^{c}	15.3 ± 7.6^{d}	6.2 ± 2.7^{e}	16.9 ± 7.1^{e}	8.7 ± 1.2^{c}
T22	62.8 ± 12.7^{b}	29.8 ± 7.5^{b}	68.6 ± 13.4^{ab}	25.7 ± 8.9^{b}	58.5 ± 11.8^{b}	48.5 ± 12.6^{b}	61.2 ± 22.5^{b}	21.5 ± 11.7^{b}
T23	72.3 ± 9.4^{ab}	32.4 ± 11.3^{b}	60.4 ± 17.9^{b}	32.3 ± 11.6^{ab}	54.9 ± 20.8^{b}	45.9 ± 9.7^{b}	58.1 ± 14.3^{b}	29.9 ± 8.3^{ab}
T24	63.9 ± 8.5^{b}	30.9 ± 7.1^{b}	59.6 ± 7.5^{b}	34.8 ± 11.2^{ab}	53.7 ± 9.4^{b}	53.2 ± 14.7^{ab}	62.4 ± 9.2^{b}	30.1 ± 5.9^{ab}
T25	60.9 ± 11.2^{b}	34.5 ± 10.8^{b}	61.9 ± 23.2^{b}	26.7 ± 7.5^{b}	57.1 ± 12.4^{b}	47.3 ± 11.9^{b}	59.8 ± 19.7^{b}	25.4 ± 10.2^{b}
T26	81.3 ± 14.2^{a}	52.4 ± 9.3^{a}	76.3 ± 15.3^{a}	48.8 ± 10.1^{a}	69.2 ± 13.9^{a}	59.5 ± 19.8^{a}	72.1 ± 21.2^{a}	39.4 ± 8.5^{a}

*Values in the table indicate means ± standard error. Columns having a common letter do not significantly differ (P > 0.05).

the lowest efficacy against dead plants in Lalehjin during 2016 was observed in T6-T9 and these were statistically at par with each other (Table 1).

The minimum infected and dead plants in Bahar and Lalehjin during 2017 were found to be in T1 and T10-T21 with statistically at par results. The highest percentage of infected plants in Bahar during 2017 was observed in T4, T6-T9 and T22-T25 and these were statistically at par with each other. In addition, the lowest effect against infected plants in Lalehjin during 2017 was observed in T5-T9 and T22-T25 with statistically similar results (Table 1).

This study indicated that the combination of biocontrol agents with low application rates of iprodione was a promising approach for controlling potato white mold. In both the years, inhibitory effect of each biocontrol agent together with application of iprodione at 60% the recommended rate was statistically similar with recommended rate. Application of formulated biocontrol agents and reduced rates of fungicides in an integrated pest management system may help in decreasing the development of fungicide resistance in pathogen population (Glare *et al*., 2012; Mallory-Smith and Retzinger, 2003).

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