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ORIGINAL ARTICLE

Integrated control of white rot disease on beans caused by *Sclerotinia sclerotiorum* using Contans® and reduced fungicides application

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Abstract This study was conducted to determine the compatibility of Contans® (*Coniothyrium minitans*) with fungicides against *Sclerotinia sclerotiorum*. Results showed that both Contans® and Topsin® significantly reduced the disease incidence caused by *S. sclerotiorum* by 90% and 95% survival plants, respectively when they were individually applied and compared to control. While, soil application of Contans® and Sumisclex mixture was the most effective in suppressing the white rot disease incidence that produced 100% survival plants, application of *C. minitans* combined with the reduced doses of fungicides would be advantageous in saving labor cost, thus increasing production efficiency of bean.

1. Introduction

White mold, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, considers a serious problem with a large number of beans (*Phaseolus vulgaris*, L.) growing under agricultural areas and low tunnels in Ismailia governorate, Egypt (Elgorban et al., 2013; Hatamleh et al., 2013). Many challenges facing the management strategies of bean white rot for instance, *S. sclerotiorum* has a wide host range reaching approximately to more than 400 species and more than 200 genera of higher plants

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(Figueirêdo et al., 2010), beside it is well known that it is very difficult to completely eradicate the tolerant reproduction structures (sclerotia) produced by this pathogen. There are many reports of serious loss due to this fungus on the bean (Partyka and Mai, 1961). Lately; this disease caused considerable yield loss in Ismailia, Egypt. An integrated disease management strategy includes biological controls, and fungicides, is highly desired to ensure efficient control of bean white rot (Clarkson et al., 2006). As high propagates of biocontrol agent are required for disease control, it is important that the bioagent is compatible with standard crop management practices including organic amendments, fertilizers and fungicides. Dicarboximide and triazole groups have provided good control of bean white rot. However, these chemicals now become less effective through inconsistent control and enhanced microbial degradation (Slade et al., 1992; Tyson et al., 1999). *Coniothyrium minitans* is considered as a promising biocontrol agent for white rot disease because it is found to effectively colonize senescent tissues (Huang, 1977), persist and spread in soil generally and in the rhizosphere specifically (McQuilken et al., 1995), in addition to suppressing the viability of new sclerotia produced on infected plants (McLaren et al., 1996; Huang et al., 2000). *C. minitans* and growth traits offer an important source in selecting and breeding for this disease. This hyperparasitic fungus can damage the sclerotia of *S. sclerotiorum* in soil plus suppressing the growth of mycelia and sclerotia in diseased crops through mycoparasitism. Soil application during solid preparations of *C. minitans* at planting time completely suppressed the production of apothecial under canopies of artificially infested host and non-host crops (McLaren et al., 1996). Spore suspension applications *C. minitans* to crops infected with Sclerotinia also decreased the apothecial production up to 90% during rotation of the crops susceptible to *S. sclerotiorum*, including carrots (Gerlagh et al., 1999). The present study aimed to evaluate the compatibility between *C. minitans* and most common fungicides used in the production of greenhouse beans in Egypt and to evaluate the effectiveness of the integrated control of *S. sclerotiorum*, which could be achieved by applying this fungus in combining with reduced fungicides application.

2. Materials and methods

2.1. Pathogenic fungus

S. sclerotiorum was used in the present study and it was isolated from sclerotia on diseased green beans (*P. vulgaris*, L.) from Ismailia governorate, Egypt. The purified fungal isolates were identified by Plant Pathology Department, Faculty of Agriculture, Mansoura University according to Kora (2003). PDA slants from the fungus were kept in 4 °C for further experiments.

2.2. Fungicides

All tested, commercial fungicides formulations were registered to be used in bean glasshouse in Egypt. Fungicides at recommended doses were used *in vivo*. The tested fungicides were as follows; Thiophanate-methyl (Topsin® M 70% WP); Mefenoxam™ (Ridomil gold®); (Captan® – 50% WP); Procymidone (Sumisclex® 50 WP); Pencycuron (Monceren® DS) and Tolclofos-methyl (Rizolex® 500 WP).

2.3. Effect of Contans® WG and fungicides on the bean white rot disease incidence caused by *S. sclerotiorum*

2.3.1. Fungi and inoculum production

Pots (30 × 25 × 30 cm) containing sterile soil (sand:loamy sand:compost, 1:2:1; 4 kg/pot) were planted with bean seeds (5 seeds/pot). Contans® WG (0.01 g/pot, each gram containing 1×10^9 cfu/g) equivalent to recommended field rate application (4 kg/ha) was added into potting soil 5 days before sowing. *S. sclerotiorum* inoculum used for this experiment was produced by autoclaving 25 g of wheat grains and 50 ml of water in 250-ml flasks for 20 min at 121 °C, then inoculating each flask with three disks (3-mm) agar plugs cut from 5 days old *S. sclerotiorum* culture then all flasks were incubated at 20 °C for three weeks before use as a source of infection. The inoculum was added at 1% (w/w) to pots after 48 h from Contans® application. Pots were arranged in a glasshouse in complete randomized design with four replicates. Treatments were as follows; non-infested, infested, Contans®, Topsin® M 70, Ridomil gold®, Captan® – 50% WP, Sumisclex® 50 WP, Monceren® DS and Rizolex® 500 WP, the fungicide used in this phase of the study was applied with the manufacturer's recommended dose (powder diluted in water). All fungicides applied as soil drenching 5 days after sowing.

2.4. Influence of using combination of Contans® WG and low doses of fungicides on white rot development in bean plants under greenhouse conditions

Plastic pots (30 × 25 × 30 cm) were filled with sandy loam soil and infested with *S. sclerotiorum* as previously mentioned. Contans® WG was applied as mixing the product with the surface layer of soil equivalent to recommended field rate (4 kg/ha). All fungicides treatments were applied as soil drenching at one or two times (0.5 g/l). Each fungicide treatment was applied directly to the soil using a plastic 1 L trigger bottle with a spray nozzle. Two control treatments were used: one of them was just water without fungicide and another one was absolute control. Each pot was placed in a separate drip tray, which was positioned on a glasshouse bench using a randomized complete block design and 0.5 cm of vermiculite was added to the top of each pot to prevent excessive drying of the soil. The glasshouse temperature was monitored and it was set at 20 ± 2 °C. Pots were irrigated as necessary. The number of survival healthy plants was recorded after 15, 30, 45 and 60 days. Influence of low doses of fungicides on the viability of Contans® WG was evaluated by taking soil samples from all treatments after 15, 30, 45 and 60 days to determinate the population of *C. minitans* in the soil according to a colony forming unit (cfu) assay method described by Whipps et al. (1989).

2.5. Statistical analysis

Data collected were statistically analyzed by the Statistic Analysis System Package (SAS institute, Cary, NC, USA). Differences between treatments were studied using Fisher's Least Significant Difference (LSD) test and Duncan's Multiple

Range Lest (Duncan, 1955). All analysis were performed at $P = 5\%$ level.

3. Results

3.1. Effect of Contans® and fungicides on the white rot disease incidence caused by ***S. sclerotiorum***

Data in Table. 1 showed that the Topsin® M 70 and Contans® significantly inhibited the disease incidence caused by *S. sclerotiorum* that produced 95% and 90% survival plants, respectively when compared to absolute (100%) and infested control (55%). This was followed by Rizolex 500 WP® which gave 85% survival plants. Conversely, Sumisclex 50 WP and Ridomil gold® were less effective on the disease incidence that giving 75% survival plants, in both fungicides.

3.2. Effect of combination of Contans® and low doses from fungicides on disease incidence caused by S. sclerotiorum under greenhouse conditions

Contans® and Sumisclex 50 WP mixture was the most effective in suppressing the white rot disease incidence that produced 100% living plants when compared to controls, followed by Contans® and Rizolex 500 WP, Contans® and Captan – 50% WP besides Contans® and Ridomil gold® mixtures that produced 90% in all mixtures mentioned. While, the lowest effect came from the mixture of Contans® and Topsin® M 70 with 75% living plants when compared with controls (Table 2).

3.3. Effect of low doses of tested fungicides on survival of C. minitans in soil

From Table. 3, it can be noticed that *C. minitans* was least sensitive to Sumisclex 50 WP that produced 1.40×10^7 cfu/g after 15 days from treating when control reach to 1.60×10^7 cfu/g and reach to 6.66×10^8 cfu/g after 60 days from treatment when control was 1.23×10^9 cfu/g. This was followed by Rizolex 500 WP with 1.30×10^6 cfu/g after 15 days from treating and 4.56×10^8 cfu/g. Alternatively, *C. minitans* was highly sensitive to Topsin® M 70 which produced 5.20×10^4 cfu/g after 15 day from treating when compared with untreated control (1.60×10^7 cfu/g), while it produced 9.43×10^6 cfu/g after 60 days.

4. Discussion

The main objective of this study was to determine the compatibility between the commercial product, Contans® and low doses of tested fungi when they were applied together and the reflection of that on controlling the white rot of green beans. The results revealed that Contans® and the fungicides alone or/and Contans® combination with low doses from fungicides investigated could suppress the white rot disease incidence caused by *S. sclerotiorum*. Also, the mixture of Sumisclex 50 WP at low dose and Contans® could decrease the disease incidence and did not inhibit the ability of Contans® to suppress the disease incidence. These results suggest that use of Contans® to manage *S. sclerotiorum* is compatible with the application of some fungicides in cultivation of beans in Egypt. Therefore, it should be feasible to apply the commercial product with low dose from fungicides to soil during seed sowing.

Combined applications of Contans® and fungicides at low doses are advantageous in saving labor costs, thereby increasing the efficiency of the production of beans. Furthermore *C. minitans* requires the attraction to and parasitism of sclerotia in soil for optimal survival. Parasitism and attraction to sclerotia in soil is mainly dependent upon stimulants exuded from sclerotia which release more exudates (Grenadine and Marciano, 1999; Partridge et al., 2006). Thus, the potential of *C. minitans* for effective attraction to and parasitism of sclerotia of *S. sclerotiorum* through exudates may be higher at the recommended rates of Contans® (Yang et al., 2011), even considering exposure to high temperatures and low *C. minitans* survivability. Furthermore, the direct interaction between sclerotia and *C. minitans* spores is very serious for infection (Budge and Whipps, 1991; Partridge et al., 2006).

Synergism between diverse biocontrol agents (BCAs) and their harmony with synesthetic fungicides was often noticed as a successful means for their combination with the current disease management practices. Combined application of *Pseudomonas fluorescens* and Trichoderma *viride* resulted in

Table 1 Effect of Contans® and fungicides on the white rot disease incidence of bean caused by *Sclerotinia sclerotiorum*.

Treatments	After 15 days			After 30 days			After 45 days			After 60 days		
	NO.	MO.%	Sur.%	NO.	MO.%	Sur.%	NO.	MO.%	Sur.%	NO.	MO.%	Sur.%
Non-infested	5.0^{a}	0.0	100.0	5.0^{a}	0.0	100.0	5.0^{a}	0.0	100.0	5.0^{a}	0.0	100.0
Infested	3.2^{c}	36.0	64.0	2.8^{c}	8.0	56.0	2.8^{c}	44.0	56.0	2.4^{c}	8.0	48.0
Contans® alone	4.6^{ab}	8.0	92.0	4.4^{ab}	4.0	88.0	4.4^{ab}	12.0	88.0	4.2^{ab}	4.0	84.0
Topsin M-70	5.0^{a}	0.0	100.0	5.0^{a}	0.0	100.0	4.8^{a}	4.0	96.0	4.8^{ab}	0.0	96.0
Ridomil	4.2^{b}	16.0	84.0	4.0^{b}	4.0	80.0	3.8^{b}	24.0	76.0	3.8^{b}	0.0	76.0
Captan	4.6^{ab}	8.0	92.0	4.6^{ab}	0.0	92.0	4.4^{ab}	12.0	88.0	4.2^{ab}	4.0	84.0
Sumisclex	4.6^{ab}	8.0	92.0	4.6^{ab}	0.0	92.0	4.2^{ab}	16.0	84.0	4.0^{ab}	4.0	80.0
Monceren	4.6^{ab}	8.0	92.0	4.6^{ab}	0.0	92.0	4.6^{ab}	8.0	92.0	4.2^{ab}	8.0	84.0
Rizolex	4.8^{ab}	4.0	96.0	4.8^{a}	0.0	96.0	4.6^{ab}	8.0	92.0	4.4^{ab}	4.0	88.0
LSD at 5%	0.42			0.40			0.47			0.59		

NO. = number of living plants. MO.% = mortality percentage. Sur.% = survival plants %.
Values within a column followed by the same letter are not significantly different according to Duncun's multiple range test ($P = 0.05$).

Table 2 Effect of Contans® and low doses of fungicides mixtures on the white rot disease incidence of bean caused by *S. sclerotiorum*.

	After 15 days			After 30 days			After 45 days			After 60 days		
	NO.	MO.%	Sur.%	NO.	MO.%	Sur.%	NO.	MO.%	Sur.%	NO.	MO.%	Sur.%
Non-infested	5.0[a]	0.0	100.0	5.0[a]	0.0	100.0	5.0[a]	0.0	100.0	5.0[a]	0.0	100.0
Infested	3.2[c]	36.0	64.0	2.8[c]	8.0	56.0	2.8[c]	44.0	56.0	2.4[c]	8.0	48.0
Contans® alone	4.6[ab]	8.0	92.0	4.4[ab]	4.0	88.0	4.4a[b]	12.0	88.0	4.2[ab]	4.0	84.0
Mixture of Contans and low dose of												
Topsin M-70	4.0[b]	20.0	80.0	3.8[b]	4.0	76.0	3.8[b]	24.0	76.0	3.8[b]	0.0	76.0
Ridomil	4.8[a]	4.0	96.0	4.8[a]	0.0	96.0	4.6[a]	8.0	92.0	4.6[ab]	0.0	92.0
Captan	4.6[ab]	8.0	92.0	4.6[a]	0.0	92.0	4.6[a]	8.0	92.0	4.6[ab]	0.0	92.0
Sumisclex	5.0[a]	0.0	100.0	5.0[a]	0.0	100.0	5.0[a]	0.0	100.0	5.0[a]	0.0	100.0
Monceren	4.0[b]	20.0	80.0	3.8[b]	4.0	76.0	3.8[b]	24.0	76.0	3.8[b]	0.0	76.0
Rizolex	5.0[a]	0.0	100.0	4.8[a]	4.0	96.0	4.8[a]	4.0	96.0	4.8[a]	0.0	96.0
LSD at 5%	0.43			0.44			045			0.49		

NO. = number of living plants. MO.% = mortality percentage. Sur.% = survival plants %.
Values within a column followed by the same letter are not significantly different according to Duncun's multiple range test (P = 0.05).

Table 3 Effect of some fungicides on viability of *C. minitans* in soil (cfu/g).

Fungicides	Days after treating			
	After 15	After 30	After 45	After 60
Non-treated	1.60×10^7	6.70×10^7	1.37×10^8	1.23×10^9
Rizolex	1.30×10^7	6.00×10^7	1.13×10^8	4.56×10^8
Topsin M-70	5.20×10^5	2.30×10^6	8.20×10^6	9.43×10^6
Sumisclex	1.40×10^7	6.16×10^7	1.16×10^8	6.66×10^8
Moncerene	9.66×10^6	1.16×10^6	1.38×10^6	9.57×10^5
Ridomil	1.16×10^7	3.30×10^4	8.53×10^7	4.64×10^7
Captan	1.20×10^7	4.26×10^7	1.02×10^8	1.20×10^8

improved biocontrol strategy than the combined application of *P. fluorescens* and thiram®. A combinatorial utility of various BCAs for enhanced and stable BCAs controlling a complex of diseases was observed (Jetiyanon and Kloepper, 2002). The present study confirmed the earlier studies that a mixture of BCAs with several mechanisms of disease management will have an additive effect and result in improved disease management compared to their individual application (Guetsky et al., 2002). Our study identified additional BCAs for control of white rot disease on *P. vulgaris*, which can be readily and stably integrated into the current production practices.

5. Conclusion

In summary, results from this study clearly demonstrated that the management of bean white rot disease caused by *S. sclerotiorum* is possible using the commercial product from *C. minitans* combined with low doses from fungicides.

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