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Integrated Effect of Biological and Chemical Control on Sclerotial Viability of *Sclerotinia sclerotiorum* (Lib.) de Bary

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ABSTRACT

Integrated effect of biocides and fungicides on sclerotial germination of *Sclerotinia sclerotiorum* (Lib.) was studied under *in vitro* conditions. MBC and Bayleton reduced sclerotial germination with an increase in dipping duration at higher concentration. At 100 μg/ml, ten and twenty min. dipping in MBC and Bayleton respectively, caused complete inhibition of sclerotial germination. Complete kill of sclerotia was obtained with MBC at 5 μg/ml with 10 min. dip and $4x10^8$ spores/ml of *Trichoderma harzianum* Rifai whereas 15 min. dip was required for the same effect with Bayleton. A combination of presoaking, high temperature, treatment with MBC and Bayleton and three biocontrol agents significantly reduced sclerotial viability. Complete inhibition of sclerotial germination was obtained with *T. harzianum* and *Gliocladium roseum* with the highest spore density ($4x10^8$ spores/ml) at 50 and 55°C.

KEY WORDS : *Sclerotinia sclerotiorum, Trichoderma harzianum, Gliocladium roseum,* MBC, Bayleton.

Sclerotinia sclerotiorum (Lib.) de Bary is a soil borne pathogen with a wide host range and is able to survive in soil for long periods as sclerotia (Purdy, 1979). The high pathogenicity of this fungus under favourable conditions and the ability of its sclerotia to withstand adverse conditions make it a highly successful pathogen. Integration of biological and chemical control seems to be a very promising way of controlling plant pathogens with minimum interferance with biological equilibrium (Henis and Chet, 1975). The present studies were, therefore, undertaken primarily to evaluate the effect of biocides and fungicides at lethal and sub lethal doses and combined effect of high temperature and presoaking treatment with fungicides and biocontrol agents on the viability of sclerotia of *S. sclerotiorum*.

MATERIALS AND METHODS

S. sclerotiorum was isolated from diseased pea plants on PDA and sclerotia were produced on sterilized oat grains at 22±1°C. Seven concentrations *viz.*, 5, 10, 15, 20, 25, 50 and 100 μg/ml of MBC 50 wp (Methyl benzimidazole carbamate) and Bayleton 25 wp(triadimefon) were used with four dipping durations of sclerotia *viz.*, 5,10,15 and 20 min. were dipped in spore suspensions of *Trichoderma viride* Persifr, *T. harzianum* Rifai and *Gliocladium roseum* each at $4x10^8$, $4x10^6$ and $4x10^4$ spores/ml for 1 h. The treated sclerotia were then washed with sterile water, dried on sterile filter paper and plated on PDA culture plates. Sclerotial germination was recorded after 96 h of incubation at 22±1°C.

For combined effect of soaking, heat treatment, fungicides and biocontrol agents, sclerotia were soaked in sterile water for 4 h and then subjected to temperatures of 40,45,50 and 55°C for 10 seconds in a thermostatically controlled water bath. Treated sclerotia were dipped in MBC 50 WP (methyl benzimidazole carbamate) and Bayleton 25 WP (triadimefon) (1 μg/ml) for 15 min. These sclerotia were then dipped in spore suspensions ($4x10^8$, $4x10^6$ and $4x10^4$ spores/ml) of *T. harzianum, T. viride* and *G. roseum* for 1 h. The sclerotia were washed with sterile water 5-6 times and plated on PDA culture plates.

Table 1. Germinations of sclerotia of *S.sclerotiorum* dipped in MBC and Bayleton suspensions for various durations [1]

Fungicide conc. (μg/ml)	MBC				Bayleton			
	Treatment Duration (Min)				Treatment Duration (Min)			
	5	10	15	20	5	10	15	20
0	83.00	82.75	87.00	78.75	81.75	83.00	78.00	80.50
5	37.50	31.00	29.75	21.50	44.25	36.00	29.75	26.25
10	33.75	29.00	21.00	17.50	37.50	31.25	25.00	20.25
15	29.50	22.50	21.00	17.50	34.75	31.00	23.75	19.25
20	23.50	22.00	17.75	9.75	32.50	29.75	21.75	17.75
25	21.00	13.25	8.25	4.75	31.50	25.75	19.00	10.00
50	7.75	4.00	0	0	18.50	12.00	5.00	0
100	1.75	0	0	0	10.75	6.25	2.75	0
S.Em ±	1.81	1.14	1.37	1.36	1.81	1.52	1.54	1.51
C.D. at 5%	5.28	3.32	3.30	3.96	5.28	4.44	4.50	4.42

[1] Average of four replications Sclerotia/treatment = 100

Each treatment was replicated four times. Heat-treated sclerotia served as check. Culture plates were incubated at 22±2°C. Germination was recorded after 96 h of incubation.

RESULTS AND DISCUSSION

The effect of different concentrations of MBC and Bayleton on germination of sclerotia of *S. sclerotiorum* indicated that at all comparable concentrations, MBC was

Table 2. Combined effect of sublethal concentrations of MBC and Bayleton and different concentrations of *T.viride* spore suspension on germination (%) of sclerotia of *T.sclerotiorum*[1]

Fungicide conc. (μg/ml)	Spore/ml	Bayleton				MBC			
		Treatment Duration (Min)				Treatment Duration (Min)			
		5	10	15	20	5	10	15	20
0	$4x10^4$	41.00	36.25	32.50	26.50	41.00	36.25	32.50	26.50
	$4x10^6$	37.25	34.50	28.50	20.00	37.25	34.50	28.50	20.00
	$4x10^8$	29.75	22.75	19.00	14.25	29.75	22.75	19.00	14.25
5	$4x10^4$	24.25	19.50	18.00	9.50	20.50	19.00	17.25	11.00
	$4x10^6$	20.50	13.75	9.00	5.75	13.75	10.25	5.75	4.50
	$4x10^8$	13.00	8.75	3.50	0	9.50	3.50	0	0
10	$4x10^4$	21.00	20.00	15.25	7.00	17.50	11.00	9.00	5.25
	$4x10^6$	13.00	12.75	10.50	4.25	10.00	6.75	3.50	0
	$4x10^8$	9.00	4.75	0	0	5.50	0	0	0
Water (control)		81.75	83.00	78.00	80.50	83.00	82.75	87.00	78.75
S.Em ±		1.55	1.36	1.43	1.24	1.57	1.24	1.47	1.23
C.D. at 5%		4.47	3.92	4.12	3.57	4.53	3.57	4.25	3.55

[1] Average of four replications Sclerotia/treatment = 100

slightly more effective than Bayleton (Table 1). However, differences in activity were generally non-significant at lower concentrations irrespective of the duration of treatment but greatly increased at higher concentrations of the fungicides, especially at 50 and 100 μg/ml. Increase in the duration of dipping in MBC at lower concentrations had only slightly greater effect on sclerotial germination. This effect was greater with Bayleton at comparable concentrations. However, reduction in sclerotial germination with an increase in dipping duration was more pronounced at higher concentrations in two fungicides. Thus, while at 100 μg/ml, MBC required only 10 min dipping for complete inhibition of sclerotial germination, 20 min dipping was required for the same effect with Bayleton.

Combined effect of sublethal concentrations of Bayleton and MBC (5 and 10 μg/ml) and different concentrations of spore suspensions of the three biocontrol agents, *T. viride, T. harzianum* and *G. roseum* on sclerotial germination of *S. sclerotiorum* revealed that biocontrol agents alone were effective in reducing the germination of sclerotia (Tables 2,3,4). Germination of sclerotia was reduced progressively and significantly with an increase in spore concentration of all the three biocontrol agents irrespective of treatment duration. The greatest reduction was observed with *T. harzianum*. Increase in the duration of treatment with spores of biocontrol agents also resulted in a progressive decrease in sclerotial germination. Pretreatment of sclerotia with sublethal concentrations of Bayleton and MBC resulted in a further decrease in sclerotial germination which again was dependent upon fungicide concentration as well as duration of treatment.

Complete inhibition of sclerotial germination was obtained at 5 μg/ml Bayleton with all the three biocontrol agents when the highest spore density ($4x10^8$ spores/ml) was used for dipping sclerotia for 20 min. However, at 10 μg/ml Bayleton and 15 min dipping, only *T. viride* and *T. harzianum* gave

Table 3. Combined effect of sublethal concentrations of MBC and Bayleton and different concentrations of *T. harzianum* spore suspension on germination (%) of sclerotia of *S. sclerotiorum*[1]

Fungicide conc. (μg/ml)	Spore/ml	Bayleton				MBC			
		Treatment Duration (Min)				Treatment Duration (Min)			
		5	10	15	20	5	10	15	20
0	$4x10^4$	38.25	35.50	28.75	24.25	38.25	35.50	28.75	24.25
	$4x10^6$	33.50	30.25	24.75	21.50	33.50	30.25	24.75	21.50
	$4x10^8$	27.50	24.50	17.00	14.00	27.50	24.50	17.00	14.00
5	$4x10^4$	20.00	15.00	14.25	11.00	17.25	13.00	14.25	7.50
	$4x10^6$	17.25	13.25	6.25	3.75	9.00	8.25	6.25	0
	$4x10^8$	9.75	6.25	0	0	4.50	0	0	0
10	$4x10^4$	16.75	14.50	8.00	3.75	15.50	11.25	6.50	4.25
	$4x10^6$	13.50	8.25	5.50	1.50	10.00	5.75	3.75	0
	$4x10^8$	10.00	7.75	0	0	2.75	0	0	0
Water (control)		81.75	83.00	78.00	80.50	83.00	82.75	87.00	78.75
S.Em ±		1.54	1.43	1.25	1.16	1.37	1.11	1.24	1.07
C.D. at 5%		4.45	4.15	3.61	3.35	3.96	3.21	3.57	3.08

[1] Average of four replications Sclerotia/treatment = 100

Table 4. Combined effect of sublethal concentrations of MBC and Bayleton and different concentrations of *G.roseum* spore suspension on germination (%) of sclerotia of *S.sclerotiorum*[1]

Fungicide conc. (μg/ml)	Spores / ml	Bayleton				MBC			
		Treatment Duration (Min)				Treatment Duration (Min)			
		5	10	15	20	5	10	15	20
0	$4x10^4$	42.25	37.50	31.25	24.75	42.25	37.50	31.25	24.75
	$4x10^6$	37.50	33.50	25.50	21.75	37.50	33.50	25.50	21.75
	$4x10^8$	30.25	28.75	21.00	16.50	30.25	28.75	21.00	16.50
5	$4x10^4$	23.50	19.50	14.75	10.25	20.00	17.00	11.75	9.00
	$4x10^6$	20.50	14.25	8.75	7.75	17.00	9.50	4.75	1.75
	$4x10^8$	11.00	6.25	3.00	0	7.50	4.25	1.50	0
10	$4x10^4$	20.00	14.50	13.75	6.25	17.75	12.50	8.00	5.00
	$4x10^6$	16.50	10.75	5.75	1.50	10.50	4.25	1.50	0
	$4x10^8$	11.25	10.00	2.75	0	7.00	2.50	0	0
Water (control)		81.75	83.00	78.00	80.50	83.00	82.75	87.00	78.75
S.Em ±		2.93	1.52	1.37	1.23	1.44	1.15	1.29	1.14
C.D. at 5%		8.45	4.39	3.96	3.55	4.17	3.33	3.72	3.29

[1] Average of four replications Sclerotia/treatment = 100

complete inhibition of sclerotial germination indicating their superior potential for biocontrol. Of the three biocontrol agents, *T. harzianum* was superior, since complete inhibition of sclerotial germination was obtained with 5 μg/ml Bayleton and 15 min dipping (Tables 2,3,4).

MBC showed greater inhibitory activity than Bayleton. Complete inhibition of sclerotial germination was obtained at the highest spore concentration of the three biocontrol agents at both 5 and 10 μg/ml MBC and even with 10 min dip in case of *T. harzianum* although for *T. viride,* this was achieved only at 10 μg/ml. Thus, MBC at 5 μg/ml with 10 min dip and $4x10^8$ spores/ml of *T. harzianum* were required for complete kill of sclerotia of *S. sclerotiorum.*

Combined effect of physical factors, fungicides and biocontrol agents revealed that exposure of presoaked sclerotia to high temperature for 10 seconds followed by treatment with MBC and Bayleton (1 μg/ml) and the three biocontrol agents reduced sclerotial viability considerably (Table 5). Most combinations of high temperature and spore densities were significantly different from each other. The three biocontrol agents were almost equally and as effective as both fungicides at comparable spore densities and exposure to high temperature.

Bavistin, benlate and captan are among the most effective fungicides recommended for the control of *S. sclerotiorum* (Hawthorne and Jarvis, 1973; Sharma, 1987). However, their effectiveness is erratic because of the soil borne nature of the pathogen which makes contact with sclerotia, the primary unit of survival/infection by the fungicides, rather difficult. Only a small proportion of the fungicide applied, that too in a diluted concentration may reach the sclerotia buried in soil which may be ineffective in rendering the sclerotia non-viable. However, the combined effects of a fungicide and a biocontrol agent offer the possibility of enhancing the potential of each other in reducing sclerotial viability, provided the fungicide is non-deleterious to the biocontrol agent or is used at sublethal concentrations. Or alternatively, the biocontrol agent has to be resistant to the

Table 5. Effect of exposure to high temperatures on the viability (%) of presoaked sclerotia of *S.sclerotiorum* treated with MBC and Bayleton and spore suspensions of biocontrol agents[1]

Biocontrol agent	Spores/ ml	MBC				Bayleton			
		Temperature (°C)				Temperature (°C)			
		40	45	50	55	40	45	50	55
T. viride	$4x10^4$	18.00	11.25	9.50	4.25	23.25	19.25	13.50	12.75
	$4x10^6$	14.25	10.75	7.00	5.25	17.50	15.50	11.50	8.25
	$4x10^8$	8.00	5.00	2.50	0.75	11.75	10.00	6.25	2.50
T. harzianum	$4x10^4$	14.00	13.50	5.75	3.25	17.00	16.25	10.00	6.75
	$4x10^6$	11.00	10.00	2.25	1.50	13.00	9.50	4.50	2.50
	$4x10^8$	5.75	1.50	0	0	6.75	5.25	0.75	0
Gliocladium roseum	$4x10^4$	17.50	11.50	7.50	3.50	19.00	15.75	14.00	11.75
	$4x10^6$	13.00	6.25	0	0	15.75	13.25	10.00	7.00
	$4x10^8$	9.50	4.25	0	0	11.50	6.50	0	0
(control)		71.50	63.25	57.50	54.75	71.50	63.25	57.50	54.75
S.Em ±		1.35	1.42	1.13	0.91	1.62	1.42	1.29	1.21
C.D. at 5%		3.90	4.10	3.27	2.63	4.68	4.10	3.72	3.49

[1] Average of four replications Sclerotia/treatment = 100 presoaking duration = 4h

fungicide at concentrations which are effective against the pathogen. Such fungicide resistant mutants of *T. harzianum* have been reported by several workers (Upadhyay and Mukhopadhyay, 1983; Papavizas and Lewis, 1981).

In the absence of such resistant mutants of biocontrol agents, the effect of sublethal concentrations of MBC and Bayleton in combination with biocontrol agents on sclerotial viability of *S. sclerotiorum* was determined. The three biocontrol agents effectively supplemented the inhibitory effect of both MBC and Bayleton by completely inhibiting sclerotial germination at spore densities of $4x10^8$/ml with 5 and 10 µg/ml of MBC and Bayleton respectively. At these fungicide concentrations, without the antagonists, sclerotial viability was considerable high. Thus, the combined effects of 5-10 µg/ml of MBC/Bayleton and biocontrol agents are almost equal to 50-100 µg concentrations of the fungicides alone indicating good scope of using fungicides and biocontrol agents in concert.

Although sclerotia of *S. sclerotiorum* are hard structures capable of surviving for long periods under adverse conditions, high temperature and alternate drying and wetting are known to reduce sclerotial viability or their capacity for carpogenic germination (Smith, 1972 a ; Abawi and Grogan, 1975). Thus, treatments of sclerotia with fungicides and/or biocontrol agents after physical treatments such as presoaking and exposure to high temperatures for short duration are likely to be quite effective in reducing sclerotial viability. Exposure of sclerotia to high temperature alone did not reduce sclerotial viability appreciably. However, subsequent treatment with biocontrol agents especially *T. harzianum* reduced viability to a greater extent as compared to treatment of sclerotia with biocontrol agents alone, indicating the possibility of potentiating the action of biocontrol agents by attenuating the sclerotia by pre-treatment with heat. The combined effect of drying and wetting and treatment with spore suspensions of biocontrol agents in initiating the rotting of sclerotia has been demonstrated in case of *Sclerotium rolfsii* (Smith, 1972b).

Similar results were obtained by presoaking the sclerotia for various durations at normal temperature. The mechanism(s) of enhanced sensitivity of pretreated sclerotia to biocontrol agents is not known. It may be due to increased loss of nutrients from the sclerotia which may enhance the colonisation potential of the biocontrol agents (Smith 1972 c).

Treatment of sclerotia with MBC and Bayleton (1 μg/ml) in addition to physical treatments and biocontrol agents, enhanced the effectiveness of treatments in reducing sclerotial viability further. *G. roseum* was particularly effective in this regard. Thus, it is possible to reduce or even destroy sclerotial viability by a judicious mix of treatments involving heat shock, presoaking and use of biocontrol agents with the minimum amount of fungicide.

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