

Control of seedling damping off caused by *Rhizoctonia solani* and *Sclerotium rolfsii* using onion broths

Control del mal de los almácigos causado por *Rhizoctonia solani* y *Sclerotium rolfsii* con caldos de cebolla

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Abstract. Damping off is a frequent disease that kills seedlings. Cultural and biological controls are the only tools in organic crops to manage this disease, and only empirical information is available on the efficiency of plant preparations. This work evaluates the effects of fermented onion decoctions on the growth of *Rhizoctonia solani* and *Sclerotium rolfsii* and disease incidence. Broth (B) and sterilized broth (SB) were respectively obtained by boiling chopped yellow onions in water, and incubating for 14 days at room temperature, with or without subsequent sterilization. The pathogens were grown on potato dextrose agar supplemented with B and SB, diluted at 1.7, 3.3, 8.3, 16.7 and 25% (v/v). Their growth was reduced by B at 8.3, 16.7 and 25%. Production of sclerotia by *S. rolfsii* was diminished by B, but stimulated by SB. *Penicillium purpurogenum*, *P. simplicissimum* and *Aspergillus niger* obtained from B behaved as antagonistic against both pathogens, showing antibiosis, competition and hyperparasitism in dual confrontations with them. Broth and SB at 10 and 50% dilutions were sprayed on chard (*Beta vulgaris*), tomato (*Solanum lycopersicum*), pepper (*Capsicum annuum*) and eggplant (*Solanum melongena*) seedlings cultivated in pathogen-colonized soil. Damping off incidence in the B treatment at 50% dilution was markedly lower than that in SB. It is concluded that B antifungal activity depends on its dilution and mycota. Broth sprays should be considered as a tool to control damping off in low-environment-impact crop production. Further studies are needed for a complete understanding of B chemical and microbiological components, as well as their changes during fermentation.

Keywords: *Rhizoctonia solani*; *Sclerotium rolfsii*; Damping off; Onion broth; Fungi.

Resumen. El mal de los almácigos causa muerte de plántulas. Los controles cultural y biológico constituyen las únicas herramientas orgánicas para su manejo. Acerca de la eficiencia de preparaciones vegetales, sólo se encuentra información de divulgación. Este trabajo evalúa el efecto de caldos fermentados de cebolla sobre el crecimiento de *Rhizoctonia solani* y *Sclerotium rolfsii* y la incidencia de la enfermedad. Se obtuvieron caldo (B) y caldo esterilizado (SB), respectivamente, mediante hervido de trozos de cebollas y fermentación a temperatura ambiente, con o sin esterilización posterior. Se cultivaron los patógenos en APG suplementado con B y SB diluidos 1,7; 3,3; 8,3; 16,7 y 25%. Su crecimiento decreció con B al 8,3, 16,7 y 25%. La producción de esclerocios por *S. rolfsii* disminuyó con B, pero aumentó con SB. *Penicillium purpurogenum*, *P. simplicissimum* y *Aspergillus niger* aislados de B evidenciaron antibiosis, competencia e hiperparasitismo respecto de ambos patógenos. B y SB diluidos 10 y 50% fueron aplicados en almácigos de acelga (*Beta vulgaris*), tomate (*Solanum lycopersicum*), pimiento (*Capsicum annuum*) y berenjena (*Solanum melongena*) infestados. La pérdida de plántulas fue menor en el tratamiento con B al 50%, en relación al tratamiento con SB. Se concluye que la actividad antifúngica de B depende de su dilución y de su micota. La utilización de caldos de cebolla debería ser contemplada como alternativa para el manejo fitosanitario de bajo impacto ambiental. Se requiere continuar los estudios para completar el conocimiento acerca de los componentes químicos y microbiológicos de los caldos, y sus posibles cambios durante la fermentación.

Palabras clave: *Rhizoctonia solani*; *Sclerotium rolfsii*; Mal de los almácigos; Caldo de cebolla; Hongos.

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INTRODUCTION

Rhizoctonia solani J.G. Kühn [teleomorph *Thanatephorus cucumeris* (A.B. Frank) Donk] and *Sclerotium rolfsii* Sacc. [teleomorph *Athelia rolfsii* (Curzi) C.C. Tu & Kimbr.] are ubiquitous soilborne pathogens with a broad host range (Sneh et al., 1995; Sarma & Singh, 2002). They survive forming resting structures that remain free or embedded in plant debris (Shlevin et al., 2003). Mycelia developed from sclerotia initiate infection causing pre or post emergence damping off (Bellido, 1995).

Allium species have a widespread use in folk medicine (Keusgen et al., 2006). Their antimicrobial properties have been studied on human pathogens (Aala et al., 2010), food degrading fungi (Yin & Tsao, 1999) and poisonous mushrooms (Auger & Thibout, 2005). Zeidan et al. (1986) found that the inclusion of onion (*A. cepa* L.) in crop rotation significantly reduced losses caused by *S. rolfsii* in subsequent productions. Onion bulb extracts inhibited the growth of *R. solani* and *Sclerotium* sp. (Navrekar & Patil, 1986; García & Padilla, 1994; Sindhan et al., 1999).

Plant preparations are frequently used for disease protection in organic crops. Onion broth is applied by spraying the base of the plants or by soil drenches (Stoll, 1986). Although empirical information is available, it still has to be elucidated whether broth effectiveness depends on chemical or microbiological activity. Our hypotheses were that onion broths control the damping off pathogens *R. solani* and *S. rolfsii* through microbiological activity, and that their efficiency is dilution-dependent. The aims of this study were to evaluate the effect of fermented onion broth and sterilized broth on the development of *R. solani* and *S. rolfsii* and damping off incidence, and to determine optimal dilutions. In addition, broth mycobiota was explored regarding biological control of these pathogens.

MATERIALS AND METHODS

Onion broth and fungal cultures. Chopped yellow onions cv. Valcatorce INTA (Valenciana group) were boiled in water (1 kg/L) for 20 minutes and filtered through surgical gauze. Volume was adjusted to 1 L by addition of tap water. Half volume of the broth (herein called B) was transferred to an Erlenmeyer flask, and incubated for 14 days at room

temperature covered with a piece of surgical gauze. The other half (herein called SB) was incubated as described above, and then sterilized by autoclaving at 121 °C for 90 minutes. New broths were prepared for each repetition of the experiments. A sample of B and SB was chemically analyzed (Table 1). *Rhizoctonia solani* O-IM-1 and *S. rolfsii* O-DI-1 were obtained respectively from *Impatiens balsamina* L. and *Dichondra microcalyx* (Hallier f.) Fabris. Their pathogenicity on the hosts selected for this work was confirmed by inoculation (results not shown).

Agar plate tests. Broth and SB were diluted in sterilized water at 5, 10, 25, 50 and 75% (v/v). Petri dishes (110-mm-diameter) were poured with 11 mL of potato dextrose agar Oxoid (PDA), cooled to 45-50 °C, and 5.5 mL of the dilutions of B, SB or water (control) were added so that the final dilutions of B and SB in the agar medium were 1.7, 3.3, 8.3, 16.7 and 25%, respectively, with five replicates for each treatment. An 8-mm diameter disk with fungal mycelium grown on PDA was placed in the centre of each plate and incubated at 20 °C, until the controls covered the whole surface (4-6 days). Sclerotia of *S. rolfsii* were counted after 14 days of incubation, while those of *R. solani* could not, as they were inconspicuous. Colony boundaries were daily reproduced on a sheet of paper and measured using a Leaf Area Scanner. The test was performed three times.

A sample of B prepared for the second test was used to explore its microbiological components. Fungal strains from B showing any kind of interactions with the pathogens on PDA were isolated and identified to species. Dual cultures of each pathogen and each B isolate were prepared by placing two 8-mm diameter PDA disks with mycelia on the centre of PDA plates separated by 4 cm and incubated for 21 days at 20 °C. Interactions between each pathogen and each B isolate were observed under the microscope (Zeiss Axioscop, Germany) at 100x magnification. The test was performed three times for each isolate.

Greenhouse assays

Chard (*Beta vulgaris* L.). Broth and SB at dilutions of 10 and 50% v/v were tested. Soil was placed into 4.4-dm³ metal containers, sterilized by tyndallization (Stanier et al., 1970), inoculated with 0.5-cm³ plugs of *R. solani* on PDA (0.1% v/v), incubated at room temperature during 14 days (Rivera et al., 2004) and disposed in plastic trays (225 cm²). Ten seeds of chard cv. Amarilla de Lyon were sown into plastic trays containing infested soil, for each of three replicates per treatment. The soil in each tray was daily sprayed with an average of 1.6 mL of water, B or SB at dilutions of

Table 1. Chemical analysis of onion broth (B) and sterilized broth (SB).

Tabla 1. Análisis químico de caldo de cebolla (B) y caldo de cebolla esterilizado (SB).

Infusion	pH	EC	Ca	Mg	K	Na	CO ₃	HCO ₃	Cl	SO ₄	P	NO ₃
B	5.3	2.3	6.0	4.6	12.6	1.8	0.0	10.0	11.4	1.1	52.0	39.0
SB	6.4	2.7	7.0	3.9	9.0	6.4	0.0	12.8	10.0	0.9	49.5	43.0

10% and 50%. Trays were kept in polyethylene bags, at 18 °C. Damping off incidence was evaluated 6 days after emergence. Samples of diseased seedlings were randomly taken, surface disinfected by immersion in 2% (v/v) of Cl as NaOCl, during 1 minute, plated on PDA, incubated at 22 °C and examined for pathogen development. A similar assay was conducted for *S. rolf sii*. The experiment was repeated twice.

Tomato (*Solanum lycopersicum* L.), pepper (*Capsicum annuum* L.) and eggplant (*Solanum melongena* L.). Soil was prepared as previously described. Experimental units were plastic trays of 140 cm² filled with soil, either sterile or infested with *R. solani*, where tomato cv. Platense Redondo Grande, pepper cv. California Wonder or eggplant cv. Long Purple were sown (1,000 seeds/m²). The soil in each tray was daily sprayed with an average of 1 mL of water, B or sterilized SB at dilutions of 10 and 50% during the experiment. The trays were individually kept in closed polyethylene bags. The experiment consisted of 10 treatments for each host, with 5 replicates, and was repeated twice. Average air temperature in the greenhouse was 16 °C. Disease incidence was evaluated as above, 25 days after emergence, and sampling for pathogen isolation was done as described. A similar assay was conducted for *S. rolf sii*.

Experimental design and data analysis. Lab data were analyzed by a two-factor study (broth-dilution) in a completely randomized design, and by Tukey multiple comparisons or DGC test (Di Rienzo et al., 2002). Shapiro-Wilks and Levene tests, respectively (Neter et al., 1996) verified normality of error terms and constancy of error variance. The number of sclerotia was analyzed by Kruskal Wallis nonparametric method (Conover, 1995). Greenhouse assays were designed as randomized complete blocks. A two-factor study was used for data analysis. Factors were analyzed separately when their interaction was non-significant. Friedman non-parametric tests were used when homogeneity of variance could not be verified (Conover, 1995). Analysis was done by InfoStat® (FCA-UNC, Córdoba), with a significance level of 5%.

RESULTS AND DISCUSSION

Agar plate tests. Inhibition of *R. solani* by B and SB was dilution-dependent; the higher dilutions were less effective. The maximum growth of the pathogen was observed for the control, followed by SB treatments (Fig. 1). B diluted at 8.3, 16.7 and 25% was the most effective in inhibition of *R. solani*. SB was less effective, especially at 1.7%. There were no differences among SB treatments at 3.3-25%. On day two, there was neither interaction between infusions and dilutions ($p=0.41$) nor effect of dilutions on pathogen growth ($p=0.21$), which was reduced by both infusions ($p=0.02$). On the third and fourth days, interactions between infusions and dilutions were significant ($p\leq 0.0001$ and $p=0.0003$, respectively).

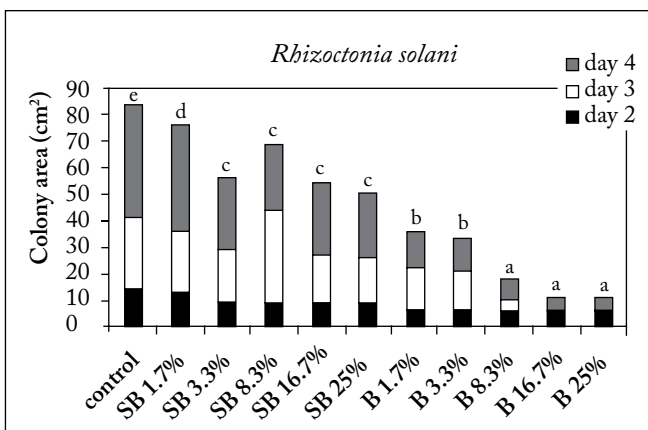


Fig. 1. Growth of *R. solani* on PDA added with sterilized (SB) and non-sterilized (B) onion broths diluted at 1.7 to 25% (v/v). Means followed by the same letters are not statistically different (Tukey test, $p\leq 0.05$). Three repeated tests.

Fig. 1. Crecimiento de *R. solani* en APG adicionado con caldo de cebolla esterilizado (SB) y no esterilizado (B) diluidos del 1,7 al 25% (v/v). Medias seguidas por la misma letra no difieren estadísticamente (prueba Tukey, $p\leq 0,05$). Tres observaciones repetidas.

Broth reduced colony growth of *S. rolf sii* at all dilutions and SB only at 25% (Fig. 2). Control was not different between B dilutions from 8.3 to 25%. SB at 1.7 to 16.7% did not differ with the control. Infusion-dilution interaction was detected in the second day of incubation ($p\leq 0.0001$). Sclerotia development was markedly reduced by B at all dilutions (Table 2). On the contrary, SB diluted at 8.3-25% stimulated the production of sclerotia.

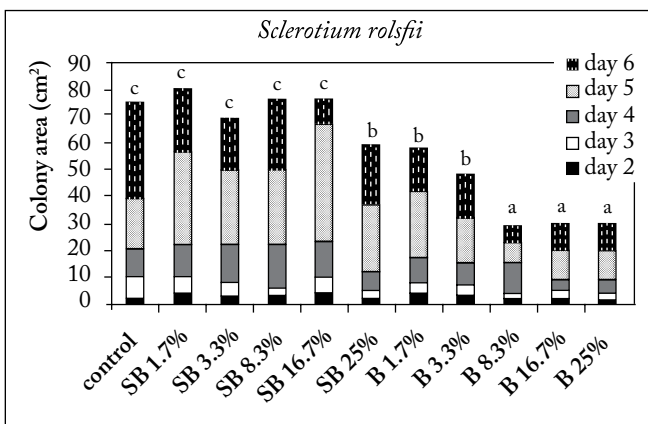


Fig. 2. Growth of *S. rolf sii* on PDA added with sterilized (SB) and non-sterilized (B) onion broths diluted at 1.7 to 25% (v/v). Means followed by the same letters are not statistically different (Tukey test, $p\leq 0.05$). Three repeated tests.

Fig. 2. Crecimiento de *S. rolf sii* en APG adicionado con caldo de cebolla (B) y caldo de cebolla esterilizado (SB) diluidos del 1,7 al 25% (v/v). Medias seguidas por la misma letra no difieren estadísticamente (prueba Tukey, $p\leq 0,5$). Tres observaciones repetidas.

Table 2. Production of sclerotia by *S. rolfsii* after 14 days of incubation on PDA supplemented with onion broth (B) and sterilized onion broth (SB).

Tabla 2. Producción de esclerocios por *S. rolfsii* luego de 14 días de incubación en APG suplementado con caldo de cebolla (B) y caldo de cebolla esterilizado (SB).

Treatment	Control	SB (%)					B (%)				
		1.7	3.3	8.3	16.7	25	1.7	3.3	8.3	16.7	25
No. sclerotia	43ef	48ef	37de	65g	66g	64g	15ab	19abc	8a	9ab	25bcd

Means followed by the same letters are not statistically different (Kruskal Wallis test, $p \leq 0.05$). Three repeated tests.

Medias seguidas por la misma letra no difieren estadísticamente (prueba Kruskal Wallis, $p \leq 0,05$). Tres observaciones repetidas.

Fungal colonies from B cultivated in PDA, inhibited *R. solani* and *S. rolfsii* or overgrew their colonies. Isolations obtained from these interactions were identified as *Penicillium simplicissimum* (Oudem.) Thom (Raper & Tom, 1968), *P. purpurogenum* Stoll (Pitt, 2000) and *Aspergillus niger* Tiegh. (Singh et al., 1991). They were stored in BAFC Cult. (FCEN Culture Collection) and labeled as 3199, 3100 and 3198, respectively.

The growth of *R. solani* was reduced in the presence of each B isolate, but this was significant only in the case of *P. simplicissimum* (Table 3, Fig. 3a). Immature sclerotia of *R. solani* were evident in the interaction with *P. simplicissimum*. Pathogen growth was inhibited in the interaction with *A. niger* (Fig. 3b) and developing sclerotia appeared in the margins of the colonies. On the reverse of the plates, *P. purpurogenum* showed an intense red pigmentation (Fig. 3c). The three B species, especially *P. simplicissimum*, grew and sporulated over the pathogen mycelium (Fig. 4a, 4b). After 14-days of incubation, *R. solani* formed brown sclerotia on the distal borders of the colony, against the three B fungi. Antagonist hyphal coiling (Fig. 4b) and pathogen necrosis (Fig. 4c) were observed for the in-

Table 3. Growth of *R. solani* and *S. rolfsii* on PDA in 7-day dual cultures with onion broth fungi and production of sclerotia by *S. rolfsii* at day 14.

Tabla 3. Crecimiento de *R. solani* y *S. rolfsii* en APG durante 7 días en cultivos duales con hongos aislados del caldo de cebolla. Producción de esclerocios por *S. rolfsii* al día 14.

Treatment	Colony area (cm ²)		No. sclerotia
	<i>R. solani</i>	<i>S. rolfsii</i>	<i>S. rolfsii</i>
Control	94.5 b	90.0 c	46 c
<i>P. simplicissimum</i>	43.4 a	24.5 a	0 a
<i>P. purpurogenum</i>	76.2 ab	49.8 b	42 c
<i>A. niger</i>	69.7 ab	36.2 a	16 b

Means followed by the same letters in a column are not statistically different (Tukey test, $p \leq 0.05$). Three repeated tests.

Medias seguidas por la misma letra dentro de la misma columna no difieren estadísticamente (prueba Tukey, $p \leq 0,05$). Tres observaciones repetidas.

teraction *R. solani* - *A. niger*. Pathogen mycelium showed markedly less pigmentation in dual cultures with *Penicillium* species, more evident for the interaction with *P. purpurogenum*.

Colony growth of *S. rolfsii* was reduced when confronted with B fungi (Table 3). On the reverse, mycelium of *P. simplicissimum* appeared discolored in contact with the pathogen (Fig. 5d). *P. purpurogenum* developed a conspicuous zone of inhibition (Fig. 5b). *A. niger* showed variable behavior, from a thin band of inhibition (Fig. 5c) to growth almost enclosing the pathogen colony. After 14 days, the production of sclerotia was similar to the control in confrontation with *P. purpurogenum*, reduced against *A. niger* and inhibited against *P. simplicissimum* (Table 3).

Greenhouse assays

Chard. Interaction between broths and dilutions was not significant for the assays with *R. solani* ($p=0.22$) and *S. rolfsii* ($p=0.55$), so the effects of both factors were analyzed separately. *Rhizoctonia* disease incidence was reduced by onion broth, diluted at 50%, (Table 5) either sterilized or not (Table 4). B was even more efficient in controlling *Sclerotium* disease (Table 4) at any dilution (Table 5).

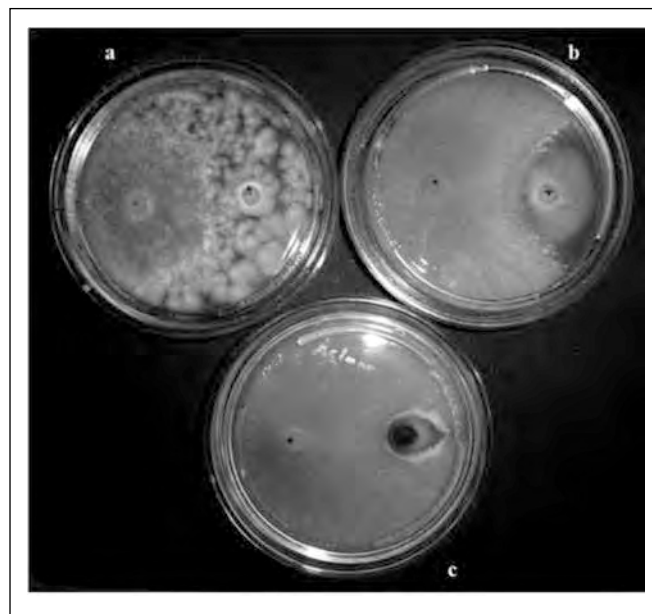


Fig. 3. Reverse growth of *R. solani* in dual cultures with (a) *P. simplicissimum*, (b) *A. niger* and (c) *P. purpurogenum*.

Fig. 3. Reverso de una colonia de *R. solani* en cultivos duales con (a) *P. simplicissimum*, (b) *A. niger* y (c) *P. purpurogenum*.

Tomato, pepper and eggplant. Damping off caused by both pathogens was controlled by B at 50% for tomato, pepper and eggplant seedlings ($p < 0.0001$) (Table 6). Broth diluted at 10% was markedly less effective to control both pathogens



Fig. 4. Interaction between *R. solani* and broth fungi. *P. simplicissimum* growing over a colony of *R. solani* on PDA (a) and under the lens (b). Hyphal coils of *A. niger* around *R. solani* (c). Hyphae of *R. solani* damaged in interaction with *A. niger* (d) (X 1,000).

Fig. 4. Interacción entre *R. solani* y hongos del caldo. Crecimiento de *P. simplicissimum* sobre una colonia de *R. solani* en APG a simple vista (a) y bajo microscopio (b). Crecimiento de *A. niger* envolviendo hifas de *R. solani* (c). Hifas de *R. solani* dañadas como consecuencia de la interacción con *A. niger* (d) (X 1.000).



Fig. 5. Growth of *S. rolfsii* (a) alone and confronted with (b) *P. purpurogenum*, (c) *A. niger* and (d) *P. simplicissimum*.

Fig. 5. Crecimiento de *S. rolfsii* en cultivo puro (a) y confrontado con *P. purpurogenum* (b), *A. niger* (c) y *P. simplicissimum* (d).

on tomato, and *R. solani* on pepper, and behaved similar to dilutions at 50% for *R. solani* on pepper and *S. rolfsii* on pepper and eggplant. Sterilized broth at 10% and 50% stimulated disease on tomato and pepper plants, respectively.

Seedling loss was observed in tomato, pepper and eggplant sown in sterilized soil irrigated with SB (Table 6). Both pathogens were re-isolated from 100% of the samples taken from seedlings that died in inoculated soil, showing necrosis at the base that made them topple over at the soil surface. General chlorosis that turned into necrosis developed on the seedlings cultivated in sterilized soil, especially those irrigated with sterilized onion broth, and no pathogens developed from those samples. Severity of leaf symptoms was similar to that observed by Aktas et al. (2006). Seedling loss in plots with sterilized soil irrigated with B diluted at 10 or 50% was low, and similar to controls.

Table 4. Diseased chard seedlings 6 days after emergence for the different sterilized (SB) and non-sterilized (B) onion broths.

Tabla 4. Plántulas de acelga enfermas a los 6 días de la emergencia para los tratamientos con caldo de cebolla esterilizado (SB) y caldo de cebolla (B).

Treatment	Incidence of damping off by <i>R. solani</i> (%)	Incidence of damping off by <i>S. rolfsii</i> (%)
Control	57 b	77 c
SB [†]	38 a	45 b
B [†]	27 a	15 a

Means followed by the same letters in a column are not statistically different (Tukey, $p \leq 0.05$). Three repeated tests. [†]Means of broth dilutions 10 and 50%.

Medias seguidas por la misma letra dentro de la misma columna no difieren estadísticamente (prueba Tukey, $p \leq 0,05$). Tres observaciones repetidas. [†]Promedios de diluciones 10 y 50% de los caldos.

Table 5. Diseased chard seedlings 6 days after emergence for the different dilutions of sterilized (B) and non-sterilized (SB) broth.

Tabla 5. Plántulas de acelga enfermas a los 6 días de la emergencia para los tratamientos con diferentes diluciones de caldo de cebolla esterilizado (SB) y caldo de cebolla (B).

Treatment	Incidence of damping off by <i>R. solani</i> (%)	Incidence of damping off by <i>S. rolfsii</i> (%)
Control	57 b	77 b
10% [†]	50 b	50 a
50% [†]	31 a	41 a

Means followed by the same letters in a column are not statistically different (Tukey test, $p \leq 0.05$). Three repeated observations. [†]Means of sterilized broth and broth.

Medias seguidas por la misma letra dentro de la misma columna no difieren estadísticamente (prueba Tukey, $p \leq 0,05$). Tres observaciones repetidas. [†]Promedios de caldo de cebolla esterilizado (SB) y caldo de cebolla (B).

Table 6. Tomato, pepper and eggplant disease incidence 25 days after emergence in pathogen infested seedling plots.
Tabla 6. Pérdida de plántulas de tomate, pimiento y berenjena 25 días luego de la emergencia en almácigos infectados.

Infusion	Soil	Mean seedling loss (%)					
		<i>Rhizoctonia solani</i>			<i>Sclerotium rolfsii</i>		
		Tomato [†]	Pepper ^{††}	Eggplant ^{††}	Tomato [†]	Pepper [†]	Eggplant [†]
Control	Sterilized	2 a	1 a	1 a	0 a	0 a	0 a
SB 10%	Sterilized	17 b	3 bc	3 bc	10 b	7 a	7 a
SB 50%	Sterilized	27 c	27 e	12 fg	20 c	19 b	19 b
B 10%	Sterilized	2 a	2 b	2 b	2 a	4 a	4 a
B 50%	Sterilized	1 a	10 d	6 d	2 a	7 a	7 a
Control	Infested	30 d	58 gh	52 h	40 d	60 d	60 d
SB 10%	Infested	32 d	62 i	57 h	48 e	60 d	60 d
SB 50%	Infested	61 e	67 i	62 h	59 f	48 c	48 c
B 10%	Infested	26 c	57 g	11 ef	21c	10 a	10 a
B 50%	Infested	2 a	35 f	9 e	4 a	8 a	8 a

Means followed by the same letters in a column are not statistically different ([†] DGC test, ^{††} Friedman test, $p \leq 0.05$). Three repeated observations. SB = sterilized broth; B = broth.

Medias seguidas por la misma letra dentro de la misma columna no difieren estadísticamente

([†] prueba DGC, ^{††} prueba Friedman, $p \leq 0,05$). Tres observaciones repetidas. SB = caldo de cebolla esterilizado; B = caldo de cebolla.

Type and dilution of onion decoctions influenced pathogen growth as well as disease incidence. In general, treatments with B were more effective than those with SB. Dilution effectiveness to manage disease differed between pathogens. These broths constitute a source of beneficial fungi that develop during fermentation, and seem to be the basis of the effect of B on disease control. It is generally assumed that correlation between lab and field studies may be low (Knudsen et al., 1997). In our case, agar plate tests gave useful information to explain greenhouse results. Even for pepper seedlings, where control of *R. solani* was less effective with B at 50%, damping off incidence could be significantly reduced from 58% (control) to 35%. Antagonistic relationships observed among the pathogens and some fungal species developed in B are based on competition, antibiosis and/or hyperparasitism phenomena. Broth controlled the growth of *R. solani* better than the growth of *S. rolfsii*. However, B isolates controlled better the growth of *S. rolfsii* than that of *R. solani*. This behavior could evidence the contribution of some other not isolated antagonist/s to B total suppressiveness or any kind of synergism. *Aspergillus* and *Penicillium* species are frequent post harvest fungi (Fatima et al., 2009; Onilude et al., 2010). *Aspergillus* is commonly isolated from onions (Zohri et al., 1993) and *A. niger* has been reported as pathogenic on them (Summer, 1995). To our knowledge, *P. simplicissimum*, *A. niger* and *P. purpurogenum* are new records as antagonists of *R. solani* and *S. rolfsii*. Among them, the first appears to be the most efficient in reducing pathogen growth and sclerotia production.

Even though this work evaluated preparations obtained from onion bulbs in relation to disease suppression, they may have nutritional value also. When compared with nutrient solutions, they are poor in Nitrogen and Calcium contents and balanced in relation to Potassium, Phosphorous and Magnesium (Hoagland & Syder, 1933); or poor in Nitrogen, rich in Potassium and balanced in other macronutrients (Hochmuth & Hochmuth, 2008). The electrical conductivity of standard solutions varies from 1.8 dS/m to 2.3 dS/m (Kayo et al., 2006; Rubio et al., 2011); and values obtained from B and SB range from 2.32 to 2.72 dS/m. Regarding pH, measured values were 5.3 and 6.4, similar to those suggested by Hochmuth & Hochmuth (2008). Broth and SB showed similar chemical composition, except for sodium content, which was markedly higher for the latter. Since salt sensitivity has been reported for Solanaceae crops, especially at the seedling stage (Aktas et al., 2006), ion toxicity may have been the cause of seedling loss in sterilized soil plots irrigated with SB.

The use of composted materials in the management of soilborne diseases has been deeply studied (Hoitink & Fahy, 1986). For instance, composted onion waste reduced viability of sclerotia of *S. cepivorum* Berk. in greenhouse pot tests (Coventry et al., 2002). In this work B behaviour was similar to those of compost teas, which are leachates obtained from composted materials (Özer & Köycü, 2006). Regarding likely mechanisms, Weltzien (1989) demonstrated that compost teas become rather ineffective after sterile filtration or heat treatments, thus attributing pathogen control to biological activ-

ity. However, in our work, SB showed some activity suggesting that chemical components have their own antifungal role.

Onions are composed mainly of water (85–90 g/100 g fresh weight). Mature intact bulbs of *Allium* species contain cysteine sulphoxides (Block et al. 1992). As in other *Allium* species (Irkin & Korukluoglu, 2007) when onion tissues are chopped the enzyme alliinase is released, converting cysteine sulphoxides into thiosulfinates (Block et al., 1992), which are reactive, volatile, odorous and lachrymatory components (Benklebia, 2004). Soil microflora is also responsible for metabolising onion compounds (Coley-Smith & Parfitt, 1986). Alliin, allicin and ajoene, the most studied sulfur-containing compounds, are thermolabile (Block et al., 1992; Sinha et al., 1992; Naganawa et al., 1996). Although antifungal activity of onion extracts decreases with increasing temperatures, Yin & Tsao (1999) found that it is still evident over 100 °C suggesting that thermoresistant components may be responsible for it. Water soluble phenols and flavones are known as important antifungal compounds expressed in onion bulbs (Link & Walker, 1933) that prevent fungal spore germination and hyphal penetration in plant tissues (Özer & Köycü, 2006). Temitope et al. (2010) demonstrated that boiling during one or two hours does not affect onion total phenol content, and diminish total flavonoid content nearly 20%. Since B and SB used in this work were obtained by boiling during 20 minutes, heat-resistant onion components like phenols and, to a lesser extent, flavonoids may have been responsible for the antifungal performance observed.

Coley-Smith & Parfitt (1986) stated that onion volatile thiols and sulphides activate sclerotia germination and growth of the onion pathogen *Sclerotium cepivorum* (Assadi & Behroozin, 1987) in the soil, possibly due to chemotaxis. In this work, it was demonstrated that onion B decreased the number of sclerotia developed by *S. roffsii*. On the contrary, SB stimulated it, which suggests any kind of unfavorable condition for pathogen's growth. It will be necessary to understand the molecular events involved in hyphal aggregation to form sclerotia (Gow & Gaad, 1995) and their requirements, to explain these effects.

Application of broth on seedling plots by irrigation, in a similar way as growers do in their fields or greenhouses, provided disease control in four different crops. As a result of this work, scientific evidence has arisen on the effectiveness of onion broth to control damping-off pathogens, and its effect was explained mainly by biological activity. As sterilized broth also showed efficiency, chemical components are supposed to have some influence on disease control. Despite the reported effectiveness against various food microorganisms, the strong odor of onions seems to have limited their use as additives or preservatives (Benklebia, 2004), but it does not seem to be an impediment for seedling protection. Further studies are needed for a complete understanding of chemical and microbiological components of the broths, as well as their changes during fermentation.

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REFERENCES

- Aala, F., U.K. Yusuf & F. Jamal (2010). In vitro antifungal activity of allicin alone and in combination with two medications against *Trichophyton rubrum*. *World Journal of Microbiology & Biotechnology* 26: 2193–2198.
- Aktas, H., K. Abak & I. Cakmak (2006). Genotypic variation in the response of pepper to salinity. *Scientia Horticulturae* 110: 260–266.
- Auger, J. & É. Thibout (2005). Sulfur compounds derived from *Allium* and crucifers and their potential applications in crop protection. In: Regnault-Roger et al. (eds), pp: 69–103. *Biopesticides of Plant Origin*. Intercept, Hampshire, USA.
- Assadi, P. & M. Behroozin (1987). The effect of bulb extracts of onion and garlic on the mycelial growth of *Fusarium* spp. and *Sclerotium cepivorum*. *Iranian Journal of Plant Pathology* 23: 1–4.
- Bedendo, J.P. (1995). Damping-off. In: Bergamin Filho et al. (eds.), pp: 820–828. *Manual de Fitopatologia*. Editora Agronômica Ceres, São Paulo, Brazil.
- Benklebia, N. (2004). Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*). *Lebensmittel-Wissenschaft & Technologie* 37: 263–268.
- Block, E., S. Naganathan, D. Putman & S.H. Zhao (1992). *Allium* chemistry: HPLC analysis of thiosulfinates from onion, garlic, wild garlic (ramsoms), leek, scallion, shallot, elephant (great-headed) garlic, chive, and chinese chive. Uniquely high allyl to methyl ratios in some garlic samples. *Journal of Agricultural and Food Chemistry* 40: 2418–2430.
- Coley-Smith, J.R. & D. Parfitt (1986). Some effects of diallyl disulphide on sclerotia of *Sclerotium cepivorum*: Possible novel control method for white rot disease of onions. *Pesticide Science* 37: 587–594.
- Conover, W.J. (1995). *Practical Nonparametric Statistics*. John Wiley & Sons, New York. 583 p.
- Coventry, E., R. Noble, A. Mead & J.M. Whipps (2002). Control of *Allium* white rot (*Sclerotium cepivorum*) with composted onion waste. *Soil Biology & Biochemistry* 34: 1037–1045.
- Di Rienzo, J.A., A.W. Guzman & F. Casanoves (2002). A multiple comparisons methods based on the distribution of the root node distance of a binary tree obtained by average linkage of the matrix of the Euclidean distances between treatment means. *Journal of Agricultural, Biological and Environmental Statistics* 7: 1–14.
- Fatima, N., H. Batool, V. Sultana, J. Ara & S. Ehteshamul-Haque (2009). Prevalence of post-harvest rot of vegetables and fruits in Karachi, Pakistan. *Pakistan Journal of Botany* 41: 3185–3190.
- García, R.P. & C.L. Padilla (1994). Evaluation of potential plant materials against some seedborne fungal pathogens of corn. *Philippine Journal of Biotechnology* 5: 159–164.
- Gow, N.A.R. & G.M. Gaad (1995). *The Growing Fungus*. Chapman & Hall, London. 473 p.
- Hoagland, D.R. & W.C. Syder (1933). Nutrition of the strawberry plant under controlled conditions. *Proceedings of the American Society for Horticultural Science* 30: 288–294.

- Hoitink, H.A.J. & P.C. Fahy (1986). Basis for the control of soil-borne plant pathogens with composts. *Annual Review of Phytopathology* 24: 93-114.
- Hochmuth, G.J. & R.C. Hochmuth (2008). Nutrient solution formulation for hydroponic (perlite, rockwool, NFT) tomatoes in Florida. Document HS796. University of Florida. IFAS Extension, Gainesville. 13 p.
- Irkin, R. & M. Korukluoglu (2007). Control of *Aspergillus niger* with garlic, onion and leek extracts. *African Journal of Biotechnology* 6: 384-387.
- Kayo, I., Y. Hiroshi & K. Akihisa (2006). Prescription of nutrient solutions suitable for the "Black Beauty" type eggplant "Kurowashi" on the rockwool facility "Kochi System" in plastic houses. *Bulletin of Kochi Agricultural Research Center* 15: 33-43.
- Keusanov, M.; R.M. Fritsch, H. Hisoriev, P.A. Kurbonova & F.O. Khassanov (2006). Wild *Allium* species (Alliaceae) used in folk medicine of Tajikistan and Uzbekistan. *Journal of Ethnobiology and Ethnomedicine* 2: 1-9.
- Knudsen, I.M.B., J. Hockenhull, D.F. Jensen & B. Gerhardson (1997). Selection of biological agents for controlling soil and seedborne diseases in the field. *European Journal of Plant Pathology* 10: 775-784.
- Link, K.P. & J.C. Walker. (1933). The isolation of catechol from pigmented onion scales and its significance in relation to disease resistance in onions. *The Journal of Biological Chemistry* 100: 379-383.
- Naganawa, R., N. Iwata, K. Ishikawa, H. Fukuda, T. Fujino & A. Suzuki (1996). Inhibition of microbial growth by ajoene, a sulfur-containing compound derived from garlic. *Applied and Environmental Microbiology* 62: 4238-4242.
- Navrekar, S.M. & B.P. Patil (1986). A study of spore germination as influenced by some plant extracts. *Journal of Maharashtra Agricultural Universities* 11: 103.
- Neter, J., M.H. Kutner, C.J. Nachtsheim & W. Wasserman (1996). Applied linear statistical models. 3rd ed. Mc. Graw Hill, Boston. 1408 p.
- Onilude, A.A., R.B. Igbinalolor & S.M. Wakil (2010). Effect of varying relative humidity on the rancidity of cashew (*Anacardium occidentale* L.) kernel oil by lipolytic organisms. *African Journal of Biotechnology* 9: 4890-4896.
- Özer, N. & N.D. Köycü (2006). The ability of plant compost leachates to control black mold (*Aspergillus niger*) and to induce the accumulation of antifungal compounds in onion following seed treatment. *BioControl* 51: 229-243.
- Pitt, J.I. (2000). A Laboratory Guide to Common *Penicillium* species. 3rd edn. CSIRO, North Ryde. 197 p.
- Raper, K.B. & C. Tom (1968). A manual of the *Penicillium* species. Hafner Publishing Company, New York. 875 p.
- Rivera, M.C., E.R. Wright, M.V. López & M.C. Fabrizio (2004). Promotion of growth and control of damping-off (*Rhizoctonia solani*) of greenhouse tomatoes amended with vermicompost. *Phyton International Journal of Experimental Botany* 53: 229-235.
- Rubio, J.S., W.E. Pereira, F. Garcia-Sanchez, L. Murillo, A.L. Garcia & V. Martinez (2011). Sweet pepper production in substrate in response to salinity, nutrient solution management and training system. *Horticultura Brasileira* 29: 275-281.
- Sarma, B.K. & U.P. Singh (2002). Variability in Indian isolates of *Sclerotium rolfsii*. *Mycologia* 94: 1051-1058.
- Shlevin, E., I.S. Saguy, Y. Mahrer & J. Katan (2003). Modeling the survival of soilborne pathogens under dry structural solarization. *Phytopathology* 93: 1247-1257.
- Sindhan, G.S, I. Indra-Hooda, R.D. Parashar & I. Hoodra (1999). Effect of some plant extracts on the vegetative growth of root rot causing fungi. *Journal of Mycology and Plant Pathology* 29: 110-111.
- Singh, K., J.C. Frisvad, U. Thrane & S.B. Mathur (1991). An illustrated manual on the identification of some seed-borne *Aspergilli*, *Fusaria*, *Penicillia* and their mycotoxins. Danish Government Institute of Seed Pathology for Developing Countries, Denmark.
- Sinha, N.K., D.E. Guyer, D.A. Gage & C.T. Lira (1992). Supercritical carbon dioxide extraction of onion flavors and their analysis by gas chromatography-mass spectrometry. *Journal of Agriculture and Food Chemistry* 40: 842-845.
- Sneh, G., S. Jabaji-Hare, S. Neate & G. Dijst (eds.) (1995). *Rhizoctonia* species: Taxonomy, molecular biology, ecology, pathology and disease control. Kluwer Academic Publishers, Dordrecht. 578 p.
- Stanier, R.Y., M. Doudoroff & E. Adelberg (1970). General microbiology. The Macmillan Press, London. 760 p.
- Stoll, G. (1986). Natural crop protection based on local farm resources in the tropics and subtropics. Josef Margraf, Aichtal. 186 p.
- Sumner, D.R. (1995). Black mold. In: H. F. Schwartz & S. K. Mohan (eds.). pp. 26-27. Compendium of onion and garlic diseases. APS Press, St. Paul, USA.
- Temitope, A.O., A.G. Olufemi & F.T. Alaba (2010). Effect of heat treatment on antioxidant activity of some spices. *Continental Journal of Food Science and Technology* 4: 53-59.
- Weltzien, H. (1989). Some effects of composted organic materials on plant health. *Agriculture, Ecosystems & Environment* 27: 439-446.
- Yin, M. & S. Tsao (1999). Inhibitory effect of seven *Allium* plants upon three *Aspergillus* species. *International Journal of Food Microbiology* 49: 49-56.
- Zeidan, P., Y. Elad, Y. Hahar & I. Chet (1986). Integrating onion in crop rotation to control *Sclerotium rolfsii*. *Plant Disease* 70: 426-428.
- Zohri, A.A., S.M. Saber & K.M. Abdel-Gawad (1993). Fungal flora and mycotoxins associated with onion (*Allium cepa* L.) in Egypt. *African Journal of Mycology and Biotechnology* 1: 74-86.