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Influence of Plant Extracts on *Sclerotium cepivorum* Development

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Abstract: There is a very limited information about plants with antifungal properties acting on roots infected by fungi. The aqueous extracts of fifteen plant species were tested against onion (*Allium* cepa) white rot fungus *Sclerotium cepivorum* that was grown in potato dextrose agar culture. Each extract presented a fungicidal effect, at a concentration of 5%, when applied on allspice (*Pimenta dioica*) and clove (*Syzygium aromaticum*). Only clove extract retained its effect at a concentration of 1%, while allspice lost it at 3%. Cinnamon (*Cinnamomum zeylanicum*) and yambean (*Pachyrhizus erosus*) extracts produced total inhibition of sclerotial production besides a poor mycelial growth. Different types of interactions were present when the extracts were mixed: all combinations presented a lost of fungicidal effect (antagonistic effect), including allspice extract; a retained fungicidal effect (single fungicidal effect) occurred in most clove mixtures and in the combination of clove and black pepper (*Piper nigrum*) the retained fungicidal effect was even below the minimal lethal dose (synergistic effect). The combination of extracts showed that the effect of each plant extract could be modified by the reactions of the complex mixture of plant compounds.

Key words: Antifungal plants, aqueous extracts, *Sclerotium cepivorum*, mycelial growth, sclerotial production

INTRODUCTION

Root diseases are a major cause of economic loss in onions and allied crops throughout the world. White rot caused by the fungus *Sclerotium cepivorum* Berk is one of the most important root diseases. The disease occurs in many areas of the world where alliums are cultivated and the environmental conditions are favorable to the pathogen. Being a soil-borne pathogen, its control measures are not easy to carry out because of its inaccessibility to fungicides and because until now resistant genotypes have not been found. In addition, biological control and cultural practices are only partially efficient (Entwistle, 1990; Schwartz and Mohan, 1995). This situation opens perspectives to test other alternatives to establish the integrated management of the disease.

In its evolutionary process plants have developed mechanisms of defense that involve, among others, the synthesis of metabolites with antifungal properties that can be used against fungi attacking humans, animals and crop plants (Rai and Mares, 2003).

The information about these plants and their antifungal metabolites on roots infected by fungi is very limited; Maruzzella and Balter (1959) found in *in vitro* tests, inhibitory action of the essential oils of *Origanum vulgare*, *Thymus vulgaris* and onions against *Fusarium oxysporum* f.sp. *conglutinans*, *F. oxysporum* f.sp. *lycopersici* and *Verticillium alboatrum*, respectively. Grainge and Ahmed (1988) reported inhibitory effects against *Sclerotium rolfsii* with extracts of *Azadirachta indica*, *Curcuma domestica*, *C. zedoaria*, *Cymbopogon* sp., *Erygeron linifolius* and *Lepidium virginicum*; Pandey and Duvey (1994) obtained good results in the control of tomato damping-off caused by *Pythium aphanidermatum* and *P. debaryanum* with essential oils of *Hyptis suaveolens*, *Muralla koenigii* and *Ocimum canum*. Bowers and Locke (2000) found that the combination of extracts of pepper and mustard, the cassia extract alone and the essential oil of clove suppress the development of *Fusarium oxysporum* in melon. Kokalis-Burelle and Rodríguez-Kabana (1994) found that growth of *Rhizoctonia solani*, *Sclerotium rolfsii*, *Fusarium oxysporum* f.sp. *vasinfectum*, *Phytophthora parasitica*, *Alternaria solani* and *Sclerotinia sclerotiorum* was reduced on agar containing either fresh or composted pine bark powder, whereas the growth of *Penicillium citrinum* was enhanced.

Regarding *Sclerotium cepivorum*, good results were obtained with the addition of *Brassica* crop residues to

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infested soil in garlic crop (Zavaleta-Mejía and Rojas, 1990); extract of *Heliopsis longipes* and their active principle affinin inhibited 100% of mycelial growth (Ramírez *et al*., 2000; Molina-Torres *et al*., 2004) and the incorporation of allyl disulfide to soil promotes the suicidal germination of sclerotia (Entwistle *et al*., 1982). The aim of the present study was to determine the influence of plant extracts from several plants species on the *Sclerotium cepivorum* development.

MATERIALS AND METHODS

Selection of plants and preparation of plant extracts: The selection of plants was based on references of antifungal plants and previous results with *Sclerotium rolfsii* (Grainge and Ahmed, 1988; Rai and Mares, 2003; González-Vargas *et al*., 2004). The plants shown in Table 1 were selected and collected in localities of the State of Morelos, Mexico. All of them were dried under shadow and milled before preparing the extracts.

Five concentrations of plant extracts, i.e., 5, 3, 2, 1, 0.5% (w/v) were prepared with distilled water and then kept at room temperature (25-28°C) for 14-16 h under continuous agitation; a control treatment without plant extracts was included. Thereafter, they were filtered and 39 g of PDA were added per liter of extract, dissolved by heating and autoclaved. Petri dishes with this mixture of synthetic media and plant extract were prepared (4 replicates/extract). Agar culture circle disks of *Sclerotium cepivorum*, isolated from garlic in Mexico, were placed in the center of each Petri dish and were incubated at 20°C. All the treatments were arranged in a completely randomized design.

Evaluation of the effect of plant extracts: The growth rate of the colony diameter was determined by measuring daily the radial growth until the fungus reached the border of the petri dish. After 2 weeks of incubation, the amount of sclerotia were determined by visual counts in every petri dish. A 5% concentration of each plant extract was tested in a first assay and in a second test the resting concentrations were evaluated. Afterwards, the plant extracts with fungicidal or fungistatic effects were combined by pairs in the same level of concentration: 1:1 (Table 4).

All experiments were repeated twice under the same conditions and the data were pooled prior analysis. All data were analyzed by ANOVA and means separated using Tukey's multiple range tests (p = 0.05).

Table 1: Plants and their organs selected for tests against *Sclerotium cepivorum*

Plant	Scientific name	Plant organs used
Alfalfa	*Medicago sativa*	Leaves, stems and flowers
Wormseed	*Teloxys ambrosioides*	Leaves, stems and flowers
Marjoram	*Origanum vulgare*	Leaves, stems and roots
Black pepper	*Piper nigrum*	Dried fruits
Allspice	*Pimenta dioica*	Dried fruits
Thyme	*Thymus vulgaris*	Leaves, stems and flowers
Cinnamon	*Cinnamomum zeylanicum*	Bark
Clove	*Syzygyum aromaticum*	Flowers
Rosemary	*Rosmarinus officinalis*	Leaves, stems and flowers
Parsley	*Petroselinium crispum*	Leaves and stems
Yam	*Pachirhyzus erosus*	Tuber root
Bougainvillea	*Bougamvillea spectabilis*	Flowers
African tulip tree	*Spathodea campanulata*	Flowers
Guamuchil	*Pithecellobium dulce*	Leaves
African marigold	*Tagetes erecta*	Flowers

RESULTS AND DISCUSSION

Effect of plant extracts at a concentration of 5% on *S. cepivorum* growth rate and sclerotial production: There were two effects on mycelial growth: total inhibition after 15 days incubation (fungicidal effect) and delayed growth rate (fungistatic effect) in relation to a control treatment (PDA without plant extract). All spice and clove showed a fungicidal effect while alfalfa, black pepper, wormseed, parsley, marjoram, cinnamon, rosemary, yambean and guamuchil reduced growth rate by more than 50% (Table 2). Several plant extracts changed color and shapes of the fungal colony after three to five days of incubation (Fig. 1). Only African tulip tree, bougainvillea, thyme and African marigold, had no effect.

There were three types of effects on sclerotial production: total inhibition of sclerotial development (cinnamon and yambean extracts), reduction of sclerotial production (bougainvillea) and significant increase of sclerotial production over the control (alfalfa, marjoram, black pepper and parsley). There were no effects on wormseed, thyme, African tulip tree and African marigold (Table 2).

Table 2: Effects of plant extracts at 5% on growth rate and sclerotial production

Plant extracts	Growth rate (mm/day)	Number of sclerotia/dish
African marigold	13.1a	136.0d
African tulip tree	13.1a	127.5d
Control	13.1a	181.5d
Thyme	11.2a	182.2d
Bougainvillea	9.8a	90.0e
Guamuchil.	5.6b	162.7d
Parsley	5.6b	538.0a
Alfalfa	5.2b	349.7b
Black pepper	5.2b	255.2c
Marjoram	5.2b	213.0c
Rosemary	5.2b	196.2d
Wormseed	5.2b	196.5d
Yam	5.2b	0.0f
Cinnamom	2.5c	0.0f
Allspice	0.0c	0.0f
Clove	0.0c	0.0f

In each column, numbers followed by the same letter are not significantly different according to Tukey´s multiple range test (p = 0.05)

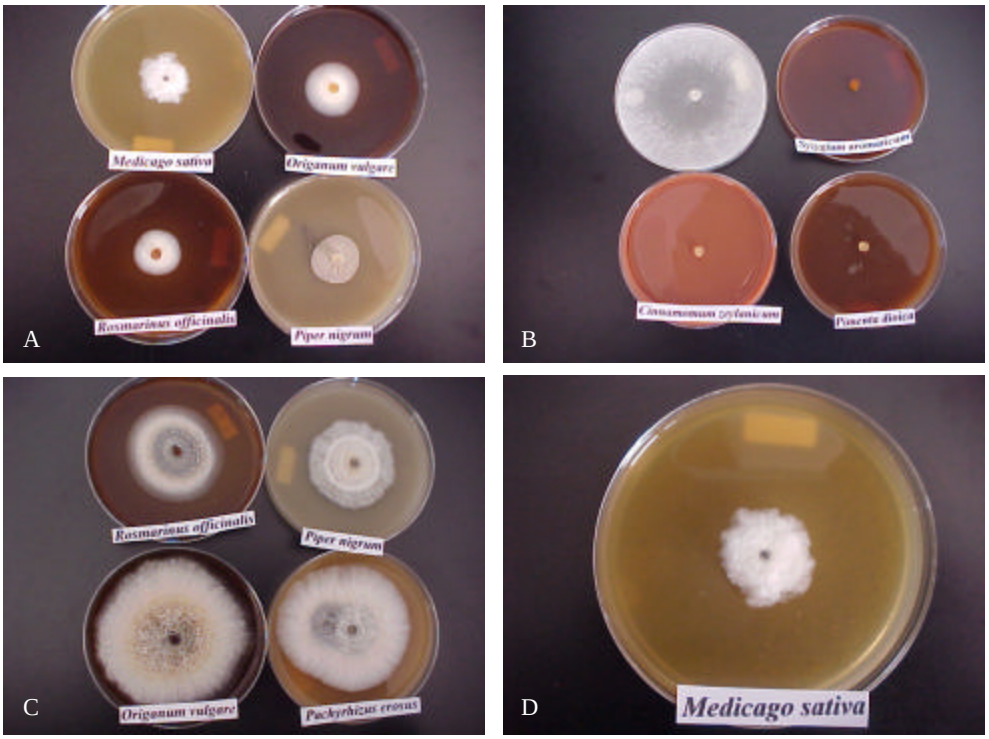

Fig. 1: Fungistatic effect on several extracts: alfalfa (*Medicago sativa*), oregano (*Origanum vulgare*), Rosemary (*Rosmarinus officinalis*) and black pepper (*Piper nigrum*) after 4 days of incubation (A) and 7 days of incubation (C) except by alfalfa and adding the effect of yambean (*Pachyrhizus erosus*). Growth absence after 4 days of incubation in the extracts of clove (*Syzygium aromaticum*), cinnamon (*Cinnamomum zeylanicum*) and allspice (*Pimenta dioica*), compared with a control with 7 days of incubation (B). Close up of mycelia affected by alfalfa (D)

All spice and clove had the same mean active principle, i.e., eugenol, but at different concentrations (Grayer and Harborne, 1994); clove produced good results with other soil-borne fungus such as *Fusarium oxysporum* (Bowers and Locke, 2000).

Since mycelium could be affected by some extracts and sclerotia by others, active principles could be different for every morphological structure. One outstanding result was that yambean and cinnamon extracts delayed mycelial growth and at the same time inhibited sclerotial development; there are no previous reports on inhibition of sclerotial production and mycelial growth reduction at the same time.

Effect of different concentrations of plant extracts: Only clove extract maintained its total inhibitory effect from 5 to 1% concentrations, while allspice lost it from 3-0.5% (Table 3). Essential oil of clove have an average yield of eugenol from 14-21% while essential oil of allspice have it from 3-4.5%, this great difference in concentration explain the efficiency of clove extract at low concentrations (Heath, 1978). Pepeljnjak *et al.* (2004) pointed out that eugenol is one of the most strongest inhibitors of enzyme processes and related compounds as methyl-or acetyleugenol could change this property.

Table 3: Effects of plant extracts at 5% on growth rate and sclerotial production

Plant extracts (%)	Growth rate (mm/day)	Number of sclerotia/dish
Allspice (3)	19.7a	139.7a
Allspice (2)	11.2a	165.7a
Allspice (1)	6.5b	103.0a
Allspice (0.5)	13.1a	153.0a
Cinnamon (3)	3.3b	76.5b
Cinnamon (2)	11.2a	102.7b
Cinnamon (1)	11.2a	116.2b
Cinnamon (0.5)	11.2a	0.0c
Clove (3)	13.1a	0.0c
Clove (2)	0.0c	0.0c
Clove (1)	0.0c	0.0c
Clove (0.5)	8.7b	170.5a
Control	19.0a	157.0a

In each column, numbers followed by the same letter are not significantly different according to Tukey´s multiple range test (p = 0.05)

Table 4: Effect of combinations between clove extract and several fungistatic extracts at different concentrations on *S. cepivorum* development

Extracts combination and concentration (%)	Growth rate (mm/day)	Number of sclerotia/dish
Clove/cinnamon (3)	3.5c	0.0c
Clove/cinnamon (2)	11.2b	162.7b
Clove/cinnamon (1)	9.8b	163.2b
Clove/cinnamon (0.5)	9.8b	0.0c
Clove/rosemary (3)	0.0c	0.0c
Clove/rosemary (2)	0.0c	0.0c
Clove/rosemary (1)	0.0c	0.0c
Clove/rosemary (0.5)	4.0b	233.0a
Clove/parsley (3)	0.0c	0.0c
Clove/parsley (2)	0.0c	0.0c
Clove/parsley (1)	0.0c	0.0c
Clove/parsley (0.5)	3.8b	130.7b
Clove/alfalfa (3)	0.0c	0.0c
Clove/alfalfa (2)	0.0c	0.0c
Clove/alfalfa (1)	0.0c	0.0c
Clove/alfalfa (0.5)	2.1b	200.7a
Clove/black pep. (3)	0.0c	0.0c
Clove/black pep (2)	0.0c	0.0c
Clove/black pep (1)	0.0c	0.0c
Clove/black pep (0.5)	0.0c	0.0c
Clove/woormse. (3)	0.0c	0.0c
Clove/woormse. (2)	0.0c	0.0c
Clove/woormse. (1)	0.0c	0.0c
Clove/woormse. (0.5)	2.1b	58.5b
Clove/yam (3)	0.0c	0.0c
Clove/yam (2)	0.0c	0.0c
Clove/yam (1)	0.0c	0.0c
Clove/yam (0.5)	3.1b	0.0c
Clove/oregano (3)	0.0c	0.0c
Clove/oregano (2)	0.0c	0.0c
Clove/oregano (1)	0.0c	0.0c
Clove/oregano (0.5)	5.0b	135.0b

In each column, numbers followed by the same letter are not significantly different according to Tukey´s multiple range test (p = 0.05)

Table 5: Effect of combinations between allspice extract and several fungistatic extracts at different concentrations on *S. cepivorum* development

Extracts combination and concentration (%)	Growth rate (mm/day)	Number of sclerotia/dish
All spice/clove (3)	0.0c	0.0c
All spice/clove (2)	0.0c	159.5b
All spice/clove (1)	6.0b	123.2b
All spice/clove (0.5)	11.2a	0.0c
All spice/rosemary (3)	7.1b	175.5b
All spice/rosemary (2)	8.7b	173.5b
All spice/rosemary (1)	8.7b	207.5a
All spice/rosemary (0.5)	5.9b	229.0a
All spice/parsley (3)	8.7b	203.0a
All spice/parsley (2)	8.7b	162.7b
All spice/parsley (1)	11.2a	200.2a
All spice/parsley (0.5)	13.1a	0.0c
All spice/alfalfa (3)	4.4b	148.7b
All spice/alfalfa (2)	7.9b	190.2a
All spice/alfalfa (1)	7.9b	252.2a
All spice/alfalfa (0.5)	9.8b	0.0c
All spice/black pep. (3)	13.1a	173.2
All spice/black pep. (2)	13.1a	140.0b
All spice/black pep. (1)	13.1a	234.0a
All spice/black pep. (0.5)	11.2a	0.0c
All spice/woormse (3)	6.1b	0.0c
All spice/woormse (2)	8.7b	0.0c
All spice/woormse (1)	9.8b	0.0c
All spice/woormse (0.5)	9.8b	205.2a
All spice/yam (3)	13.1a	0.0c
All spice/yam (2)	13.1a	146.5a
All spice/yam (1)	13.1a	0.0c
All spice/yam (0.5)	13.1a	178.2a
All spice/oregano (3)	11.2a	168.2a
All spice/orégano (2)	11.2a	196.5a
All spice/orégano (1)	13.1a	191.2a
All spice/orégano (0.5)	13.1a	79.2b
All spice/cinnamon (3)	8.7b	180.5a
All spice/cinnamon (2)	11.2a	101.7b
All spice/cinnamon (1)	13.1a	0.0
All spice/cinnamon (0.5)	13.1a	0.0
Control	19.7a	202.5a

In each column, numbers followed by the same letter are not significantly different according to Tukey´s multiple range test (p = 0.05)

Effect of combinations between fungicidal and fungistatic plant extracts: Different types of interactions were observed when the extracts were combined by pairs (Table 4 and 5): a) antagonistic effect when the combination lost the fungicidal effect of one of the extracts, this was observed in all combinations including allspice and in that of clove with cinnamon at 3 to 0.5%; b) single fungicidal effect when the combination retained the fungicidal effect, this occurred in most clove combinations with the exception of those with cinnamon and low doses of allspice; c) synergistic effect when an extract keeps its fungicidal effect even below 1% concentration (the lowest lethal dose of clove), this happened only when combined clove with black pepper. Sclerotial production was inhibited by some treatments with poor mycelial growth like clove/cinnamon 0.5%, allspice/clove 0.5% and allspice/yam 3%.

These results of combinations showed that the effect of every plant extract could be modified by the reaction between compounds in the complex mixture of plant extracts and this situation could be similar to the process that happens in the soil environment when a substance is added. It is necessary to confirm the results found in this work under greenhouse and field conditions because natural conditions might modify the response of *S. cepivorum*.

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