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Effect of vegetables extracts and synthetics fungicides on the micelial growth of in vitro *Sclerotium rolfsii* and *Thielaviopsis basicola*

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Abstract

The antifungical effect of castor extracts *Ricinus communis* L.; sweet basil *Ocimum basilicum* L.; wild peppergrass *Lepidium virginicum* L.; garlic *Allium sativum* L.; neem *Azadirachta indica* A. Juss and fungicides benomyl (Benlate) and iprodione (Rovral) was proved on the mycelium growth *in vitro* of *Sclerotium rolfsii* and *Thielaviopsis basicola*. Sodium hypochlorite at 3% and distilled water were used to disinfect and to wash the vegetal parts. For the obtaining of juicy extracts, materials were macerated, the extract filtered with Whatman Nº1 paper and then sterilized with Millipore filters of 0.45 mμ. All tested extracts and fungicides showed some inhibit activity on the mycelial growth of *Sclerotium rolfsii* and *Thielaviopsis basicola,* in a papa dextrose (PD) mean, resulting in garlic a total inhibition of grow until 7 days. Benomyl and garlic reduced the grow of *Thielaviopsis basicola* virtually to cero during 21 days. Significant differences were found with iprodione (79%), benomyl (54.1%) and castor (8.3%) in relation to the witness. The number of sclerotium was lower in treatments with garlic, neem and benomyl compare to the witness (27.6, 49.3, 68.3 sclerotium vs 143.5 sclerotium). According to these results garlic might be considered as an alternative to be used in a handle strategy of these fungi.
Key words: Vegetal extracts, fungicides, mycelial growth, *Sclerotium rolfsii*, *Thielaviopsis basicola*.

Introduction

In the last years the society has considered the environmental aspects as important, carrying lots of investigations toward the discovery of new inactive materials that might be employed in the integral handle of pest with less negative effects on the environment by being natural products (4, 5, 15). The production of bioactive substances or secondary metabolites by plants happens through different metabolic ways

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generating a huge number of compounds, many of these are only detected in a determine group of plants and in variable concentrations. The quantity and composition of this type of compounds is very variable and depends on the type of tissue, age of the plant, its habitat and type of soil (7).

Many of these compounds are produced and stored in young tissues, mainly in leaves or productive tissues such as flowers and seeds (Costa Mauro J. N. *et al.*) (6). Lots investigations have proved that some of these compounds affect important function in vegetables, since these can act in the preservation of the integrity of the plant against the attack of enemies as nematodes, bacterium, fungi, insects and herbivorous or in the attraction of pollinators and dispensers of seeds (3, 7).

Crops are constantly threatened by illnesses, originated by infectious agents that attack different organs of the plants: root, stem, leaves, inflorescence and fruits, where the severity of the damage depends on the susceptibility of these and the favorable environmental conditions that allow the fast development of phytopathogens organisms.

Most of these fungi that cause infections in plants are facultative parasite that live in the soil and some of the most destructive illnesses are caused by them. Roots of plants are the most known, specially in very wet soils, leaves, stems, fruits, seeds, cortex and central wood of trees might also be affected (1).

Trying to reduce the damage that these organisms cause to the crops the man has caused an ecological unbalance, due to trying to control them also causes damages to the regulator fauna and polluting the water, the soil and products that are consumed by the population (7, 13, 21).

Among pathogens that cause these damages are fungi of the *Sclerotium* and *Thielaviopsis* genre, able to originate lost in the production due to damages that cause to different crops (1, 12).

S. rolfsii might cause a withered state and rotting in hosts. The fungus might survive as a sclerotium in the soil and in the rest of the crop by a long period, being this the main source of inoculum for the following harvest (11). Black rotting of the root caused by *T. basicola* is well distributed, affecting vegetables, some annual crops and fruits, occasioning important lost in fresh temperature conditions and high humidity in the soil (1).

The diverse methods of available control, specially the chemical control, have problems regarding its efficiency, cost and negative impact to the environment (12).

Due to this situation, other alternatives are being searched for controlling these fungi in the field, as for example the biological control which main property is the ecological stability. This offers high perspective to minimize the effect of illnesses in the agriculture production without worsen problems of environmental pollution that threatened the ecological balance in the present and in the immediate future (4, 7, 22).

Several investigations have been done worldwide showing the antifungal action of some vegetal extracts in the foliar level as well as in the soil (2, 4, 5, 14, 16, 17, 19, 20, 22).

In the evaluation *in vitro* of the effect of juicy extracts and neem oil (Azadirachta indica) on *Sclerotium rolfsii*, Singh *et al*. (17) observed that the growth of the fungi was inhibited completely when neem oil was used in concentrations of 1.500, 2.000 and 5.000 ppm. When the extract of the pulp of the fruit was used, the required concentration to inhibit growth was of 12.000 ppm and in the case of leaves extracts the completed inhibition of the fungi growth was achieved with the use of 10.000 ppm.

Delp and Klopping (8) report the use of benomyl in the control of *S. rolfsii*, but with very poor effects. On the other hand Edginton *et al*. (9) mention benomyl as highly toxic for *T. basicola, in vitro*, with LDs of 0.2 ppm.

Though of existing in Venezuela a huge quantity of species of plants, investigations oriented to detect and understand the effects of substances consequence of the secondary metabolism on fungi are scarce. In this matter, the aim of this research is to evaluate *in vitro* the effect of different vegetal extracts and chemical products on the mycelial growth of fungi *Sclerotium rolfsii* and *Thielaviopsis basicola*.

Materials and methods

Preparation of extracts

Samples of fresh leaves of wild peppergrass *Lepidium virginicum* L., and sweet basil *Ocimum basilicum* L.; macerated seeds of neem *Azadirachta índica* A. Juss. and of castor *Ricinus communis* L. and peeled garlic *Allium sativum* L., previously disinfected externally with sodium hypochlorite at 3% as washed with distilled sterile water. The obtained extract was filtered with the filter Whatman Nº 1 and sterile by filtration with Millipore filters of 0.45 mμ .

Synthetic fungicides were diluted in distilled sterile water using doses of 2.5 g of benomyl P.C and 3.0 g of iprodione P.C by liter of water.

Obtaining of pathogens

With the purpose of obtaining the necessary material for the preparation of treatments strains of *S. rolfsii* and *T. basicola* were used, from the phytopathogens collection of CIAE Lara, which grew for 15 days in petri plates with agar-papa-dextrose (APD) on laboratory conditions (22ºC – 29ºC).

Treatments

Through plates previously prepared mature sclerotium were selected in S. rolfsii, putting 3 sclerotium in each tube that had 10 ml of liquid mean papa-dextrose (PD), poured with a mean dispenser and 5 ml of each extract or chemical product, added with a pipette. For fungus *T. basícola,* 1 ml of a juicy substance of 4-5 x 10^5 conidia/ml was added to each tube that had 10 ml of the PD mean and 5 ml of each extract or chemical product.

The evaluated treatments were: 1) papa dextrose (PD) + distilled sterile water (witness); 2) papa dextrose (PD) + juicy extract of sweet basil leaves (30g/l); 3) papa dextrose (PD) + juicy extract of neem seeds (30g/l); 4) papa dextrose (PD) + juicy extract of garlic (5g/l); 5) papa dextrose (PD) + juicy extract of wild peppergrass (30g/l); 6) papa dextrose (PD) + juicy extract of castor seeds (30g/l); 7) papa dextrose (PD) + benomyl (2.5g/l) and 8) papa dextrose (PD) + iprodione (3.0 g/l) with three tubes per essay and three replications for a total of 72 tubes.

According to the methodology proposed by Singh *et al* (17), the determination of the fungus growth was done at 7, 14 and 21 days of sowed in the PD mean. For this reason, treatments were filtered with Whatman Nº 1 filters, the mycelium of the fungus was let dry for 24 h at 70ºC in a stove and was weighted. The number of structures of resistance or sclerotium formed in each tube were also counted in the case of *S. rolfsii* in the different treatments and for *T. bassicola,* the germination counting of conidia was done in each treatments with the help of a Neubawer camera.

A completely randomized arrangement was used, and for the analysis the transformation of the data was done using the formula Ö 1 + x and the mean comparison according to Newman Keuls at 5%.

Results and discussion

In figure 1 is shown the growth of the fungus *Sclerotium rolfsii* in the papa dextrose (PD) means, with natural extracts and synthetic fungicides.

According to these results it is observed that passed 7 days all extracts and fungicides inhibited or delayed the grow of the fungus compare to the witness treatment; resulting in the case of garlic a total inhibition of the grow of it during that period.

These results agree to those obtain by other authors who have mentioned the inhibit effect of garlic extract on the grow of different fungus as: *Rhizoctonia solani, Alternaria spp* and *Curvularia spp,* using variable concentrations between 8 and 50 g/l (2, 10).

The inhibitor effect of the vegetal extracts and fungicides on the growth of *S. rolfsii* was variable in the time. When the effects of the vegetal extracts that caused a higher inhibition of the grow of *S. rolfsii* (wild peppergrass, neem, garlic) are compared in relation to other fungicides, can be observed that these last were only surpassed at the beginning of the experiment (7 days), reducing since that moment the inhibitory capacity of extracts. This might suggest the necessity of using higher concentrations with the aim of prolonging its effect (figure 1).

The short inhibition period of *S. rolfsii* in the case of garlic extract might be related to the low concentration used (5 g/l) compare to concentrations of 8, 10 and 50 g/l used in other investigations where this product has been evaluated as fungitoxic (2, 10); nevertheless, at 14 days when this inhibitor effect on the grow of the fungus finishes, it is evidenced a higher growth of the witness. On the other hand, in the case of neem, results might be related to the concentration of the oil extracted from the seed in the obtained mix as a consequence of the extraction method used, more efficient results have been obtained with the use of oil of higher pureness and concentration using a Soxhlet equipment to obtain it (17).

Passed 21 days of the experiment was observed a growth in iprodione, benomyl and castor treatments, with an inhibition of 79.1%, 54.1% and 8.3% respectively, however, castor did not show significant differences with the witness nor with neem, wild peppergrass and sweet basil treatments (figure 1).

In general terms, the inhibitory effect of the *S. rolfsii* effect by different vegetal extracts and synthetic fungicides reduced in the time, observing in these specific cases a higher effect on the grow reduction by iprodione compare to benomyl, these results do not agree to the observations done by Delp and Klopping (8) who report a poor effect of Benomyl on *S. rolfsii* (figure 1).

The information regarding the average of mature sclerotium at 21 days of the experiment shows a lower number of these in treatments with garlic, neem and benomyl (27.67, 49.33 and 68.25 sclerotium respectively) inferior to the witness (143.5) and significantly inferior to the treatment with castor (157.0). This is a very important aspect if it is

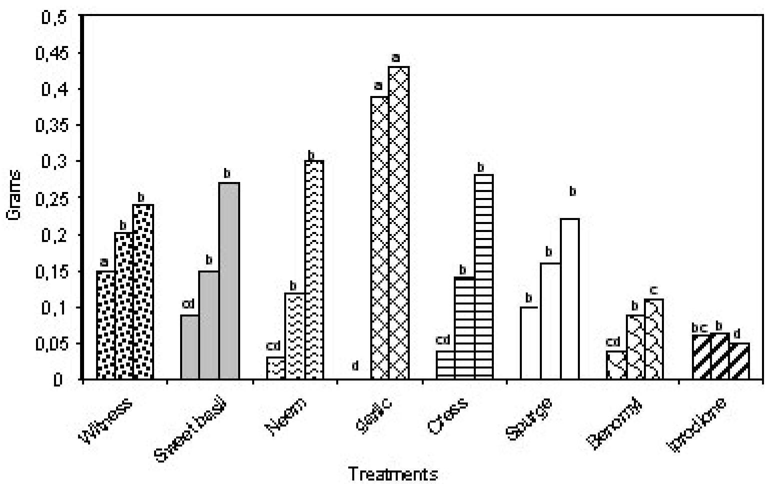

Figure 1. Mycelia growth (g) of *Sclerotium rolfsii* fungus on Papa dextrose media (PD) with vegetable extracts and synthetic fungicides.

considered that these are the resistance structures of the fungus that allow it to survive on adverse conditions, thus it is necessary to consider this aspect for giving recommendations about its proper handle, specially if it is observed that in all treatments viable sclerotium were formed (figure 2).

Figure 3 shows the growth of fungus *Thielaviopsis basicola* in the PD means with different treatments of vegetal extracts and fungicide benomyl. According to the results until 21 days, all treatments reduced the mycelial growth of the fungus compare to the witness. During the same period, the growth was virtually cero for treatments benomyl and garlic, likewise, these treatments provoked the lower germination of conidia of the fungus (figure 4). Singh (18) also observed inhibitors effects of the garlic on the germination of conidia of some species of *Fusarium* and of *Colletotrichum*, while other authors did not find any effect on the germination of conidia of *Curvularia sp* y *Alternaria sp* (2,18).

In the case of benomyl, the inhibition of the growth of *T. basicola* was almost total, these results agree to those observed in vitro by Edginton *et al.* (9), who report benomyl as highly toxic for this fungus in concentrations of even 0.2 pp (9) (figure 3). Generally, results in vitro obtained in this investigation proves the presence of different fungi-toxic substances which inhibit the growth of *S. rolfsii* and *T. basicola* in the species of plants and parts used. Further investigations are required with diverse concentrations of these products in vitro, as well as the incorporation of others and the definition of an application strategy adequate to the field, considering the characteristics of the role of each of these fungi and conditions that favor the development of illnesses caused by them.

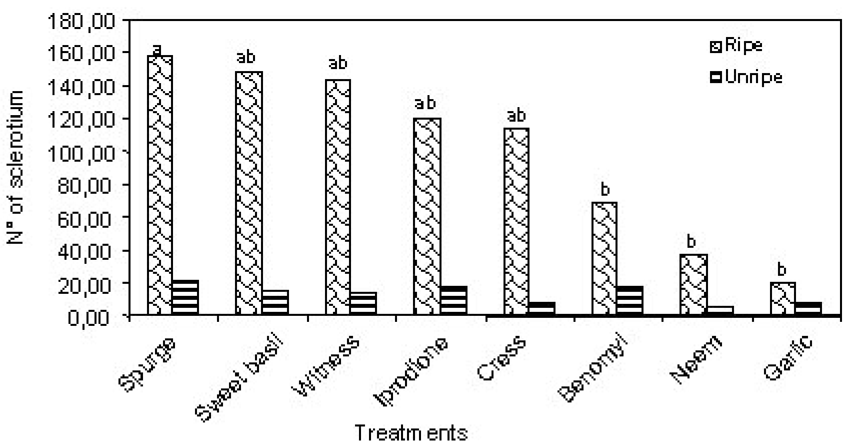

Figure 2. Formation of sclerotium of *Sclerotium rolfsii* on Papa-dextrose media (pD) with vegetable extracts and synthetic fungicides 21 after the incubation.

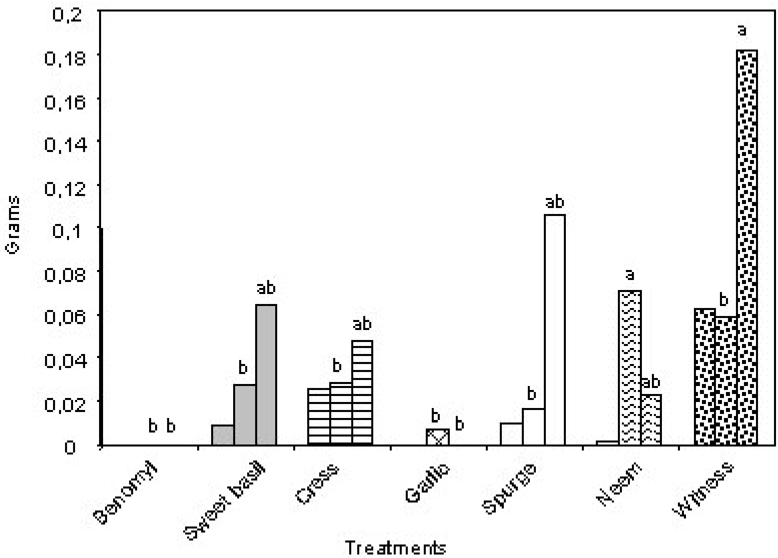

Figure 3. Mycelia growth (g) of the *Thielaviopsis basicola* on a Papa dextrose media (PD) with vegetables extracts and synthetic fungicides.

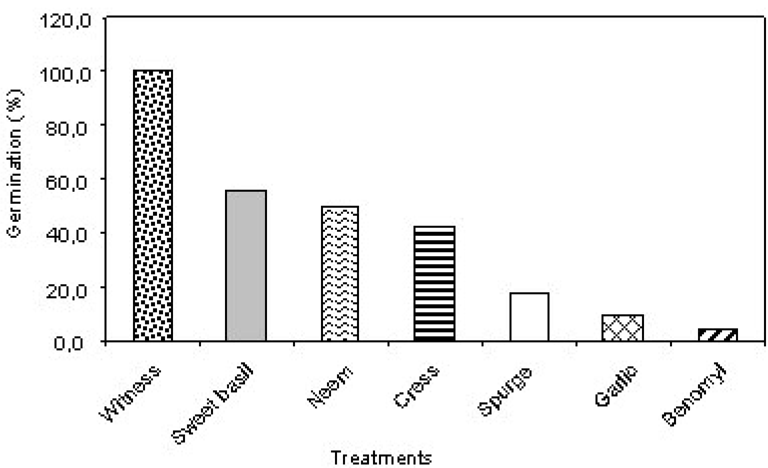

Figure 4. Germination of conidium of *Thielaviopsis basicola* fungus on Papa dextrose media (PD) with vegetables extracts and synthetic fungicides.

Conclusions

21 days after had sowed in papa dextrose (PD) mean it was found a reduction of the mycelial growth of *Sclerotium rolfsii* with iprodione, benomyl and castor extract.

The number of formed sclerotium reduced in treatments with benomyl and with neem and garlic extracts.

All treatments reduced the mycelial growth and the conidia germination of *Thielaviopsis basicola.*

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