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RAPID COMMUNICATION

Antifungal activity of *Solanum* extracts against phytopathogenic *Curvularia lunata*

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Abstract

Several species of *Solanum* produce secondary metabolites with antimicrobial activity. In the present study, the inhibitory activity of *Solanum chrysotrichum*, *S. erianthum, S. torvum* and *S. rostratum* against phytopathogenic *Curvularia lunata* was determined. Methanol extracts from roots, stems, leaves and fruits were evaluated by the method of mycelial inhibition on agar and the minimum inhibitory concentration (MIC) was determined on a liquid medium. To increase the antimicrobial activity, the combined activity of the most active extracts for each phytopathogen was also determined (a combination of intra and interspecies extracts). The results showed that 12 of the 16 methanolic extracts of *Solanum* species had antifungal effects against *C. lunata*. The extracts of *S. rostratum* and *S. erianthum* developed the highest activity (~80% inhibition and 28.4 MIC $\mu g \cdot ml^{-1}$), even, equal to or greater than, the reference fungicide. The mixture of the active extracts of *S. chrysotrichum* and *S. torvum* increased their activity. Various extracts affected the macro and microscopic morphology and most of them reduced the number of conidia of the fungus. This resulted in the capacity to control the vegetative growth and reproduction of *C. lunata,* the causal fungus of corn leaf spot disease.

Keywords: *Curvularia lunata*, botanical extracts, control plant diseases, leaf spot disease, phytopathogen inhibition, plant antifungals

Curvularia lunata (Wakker) Boed is a phytopathogenic fungus that causes the "leaf spot disease" or "*Curvularia* leaf spot" in corn, barley, rice and sorghum. It is one of the most economically devastating diseases in maize cultivars (Akram *et al.* 2014; Gao *et al.* 2014). One alternative for controlling phytopathogens is the use of plant extracts, which has led to the search for antifungal metabolites with faster rates of biodegradability (Baka 2014; Abhishek *et al.* 2015; Shabana *et al*. 2017). This activity can be achieved by antifungal specialized metabolites that can inhibit the mycelial growth or spore germination and trigger plant resistance (Bassolé and Juliani 2012).

Solanum L. (Solanaceae Juss.) is a genus of plants that produces secondary metabolites with inhibitory activity against dermatophyte and opportunistic fungi (Das *et al.* 2010; Essien *et al.* 2012; Herrera-Arellano *et al.* 2013). The inhibitory activity of the extracts and compounds against phytopathogens is less well known (Abhisheki *et al.* 2015). We report here the antifungal activity of four *Solanum* species (*Solanum chrysotrichum* Schltdl, *S. erianthum* D. Don, *S. torvum* Sw and *S. rostratum* Dunal).

Solanum species were collected in Chiapas, Mexico. The botanical voucher specimens were deposited in the Herbarium HEM at the UNICACH. Fresh plant material (roots, stems, leaves and fruits) of each species was extracted with methanol (100 g : 500 ml dissolvent) at room temperature for 24 h in the dark. Then, the extract was filtered and the plant material was re-extracted in methanol twice. The resulting filtrates were combined and the solvent was removed under reduced pressure.

Curvularia lunata was donated by the Agricultural and Biotechnology Laboratory of the Faculty of Agricultural Sciences at the Universidad Autónoma de Chiapas. To verify its identification, genomic regions

such as ITS1 and ITS4 where sequenced in the laboratory of GeMBio at the Centro de Investigación Científica de Yucatán AC (CICY).

A modified agar dilution method (Eksteen *et al.* 2001) was used for determining the inhibition of mycelial radial growth of *C. lunata*. The mycelial growth inhibition of the plant extracts was determined at 170 μg · ml^{-1} dissolved in methanol : ethanol (1 : 1) at 2.5% v/v of the culture medium. Mycelial growth was recorded every 24 h until the growth of the control (with solvent, but without extract) completely covered the surface of the culture medium (5-cm diameter). The antifungal activity was expressed as % inhibition using the following equation: [(dc–de)/dc] × 100, where: dc – fungal growth diameter without extract (negative control), de – fungal growth diameter with extract. The experiment was repeated twice with five replicates (n = 10).

For each plant species, the extracts that showed the highest antifungal activity were selected, combined (*intra*-species mixture extract), and then evaluated to determine whether their potency was additive. Furthermore, the most effective extract of each plant species was combined and evaluated (*inter*-species mixture extract). The commercial fungicide Captan® was used for positive control under the same conditions and the same concentrations of extracts.

Determination of the minimum inhibitory concentration (MIC) was performed in potato dextrose broth for 72 h at 30°C (Moussa *et al.* 2013). The extracts were dissolved in dimethyl sulfoxide at 1-% v/v, and then evaluated at serial concentrations between 900 and 1.75 μg · ml^{-1}. All determinations were made in triplicate.

Macroscopic characteristics from mycelial growth were analyzed every 24 h. For microscopic characterization, clear adhesive tape fragments of 1 cm^2 were taken and placed on the plant pathogen mycelium grown on Sabouraud Dextrose Agar (SDA). Each sample was placed on a slide and fixed with a cover slip. Observations of the shape and color of conidia and hyphae were performed under a Carl Zeiss Axiostar™ microscope at 40 and 100×. Photographs of the observations were taken (40×) with a microscope camera (AxioCam 105) and, using the ZEN 2012 program by ZEISS, a count of conidia was performed in an area of 3,705.015 μm^2 (n = 10).

The data of mycelial inhibition and conidia number by species, organ, mixtures (*intra*-species and *inter*-species) were analyzed using a non-parametric analysis of Kruskal Wallis and a multiple comparison of Mann-Whitney, performed with the statistical software Past (Hammer *et al.* 2001). For each extract, the growth rate (in mm · h^{-1}) was calculated from the slope of the equation of the linear regression model of each growth curve.

Results and Discussion

In *S. chrysotrichum,* the root extract showed the highest inhibition (56%). The stem and leaf extracts were less active and the fruit extract was practically inactive (p = 0.0001; Table 1). These activities were associated with the growth rate of the fungus. With the fruit extract, the growth rate was similar to the control without extract (0.30 mm · h^{-1}), while the growth rates of the leaf and stem extracts were 7 and 27% lower (0.28 and 0.22 mm · h^{-1}, respectively). With the root extract it was reduced to 70% (0.09 mm · h^{-1}), similar to that caused by the reference fungicide (0.08 mm · h^{-1}). In *S. erianthum* the stem extract was the most potent (77%, p = 0.00001; Table 1). Root and leaf extracts caused a reduction of growth rate by almost 50% (0.17 and 0.16 mm · h^{-1}, respectively). The fruit extract caused a reduction of growth rate of up to 70% (0.09 mm · h^{-1}) and was similar to Captan®. The stem caused a decrease of 89% in growth speed (0.034 mm · h^{-1}), which was 60% slower than the growth caused by the fungicide reference. In *S. rostratum*, fruit and leaf extracts were the most active. The stem extract provided moderate activity and the root activity, unlike the other plants, showed low potency (65, 51, 40, 32%, respectively; Table 1; p = 0.0001). The fruit extract caused a similar growth to that of Captan®. In *S. torvum,* only the root extract had prominent activity (50%), reducing the growth rate similarly to the reference fungicide (Table 1; p = 0.0001).

The mixture of *S. chrysotrichum* extracts had the highest activity in this test (65%, Table 1). It exceeded up to twice the activity of one of its individual extracts (leaf, 31% activity). *Solanum torvum* slightly increased the activity of its root extract (5% more) and significantly of the stem (20 to 56%). The *inter*species mixture had a medium activity (42%), which can be considered as a negative result since all individual extracts had higher activities than the mixture.

The extracts of *S. chrysotrichum* had similar MIC (112.5 μg · ml^{-1}) (Table 1). In *S. erianthum*, the fruit extract had twice the antifungal capability (56.25 μg · ml^{-1}) than the root, stem and leaf (112.5 μg · ml^{-1}). On the other hand, *S. rostratum* activity in the leaf extract was the most potent (28.1 μg · ml^{-1}). The stem and fruit had the same effect (112.5 μg · ml^{-1}), and the root was the least active (225 μg · ml^{-1}). The *S. torvum* stem and leaf extracts had MIC of 112.5 μg · ml^{-1}. The root extract needed twice the concentration and the fruit needed quadruple.

The extracts led to changes in color and appearance of the mycelium. Some extracts caused alterations in the microscopic morphology of *C. lunata* (Fig. 1). The root extract of *S. torvum* reduced the size and shape of the conidia (Fig. 1C). However, the intra-species mixture extract of *S. rostratum* caused greater septation in the hyphae (Fig. 1F). Some extracts and

Table 1. Antifungal activity of methanolic extracts from *Solanum* spp. against *Curvularia lunata*

Plant extract	*Solanum chrysotrichum*	*Solanum erianthum*	*Solanum rostratum*	*Solanum torvum*
	Mycelial inhibition [%]*			
Root	55.7 ± 9.2 a	50.1 ± 7.3 d	31.7 ± 8.3 d	50.42 ± 8.3 b
Stem	38.8 ± 11.7 b	77.4 ± 2.7 a	40.5 ± 8.2 cd	20.12 ± 8.8 c
Leaf	30.9 ± 9.4 b	42.4 ± 4.8 e	50.5 ± 5.2 c	2.71 ± 1.3 d
Fruit	1.8 ± 3.1 c	61.6 ± 2.8 c	64.8 ± 6.2 b	5.05 ± 4.4 d
Captan®	69.8 ± 7.9 a	69.8 ± 7.9 b	69.8 ± 7.9 a	69.8 ± 7.9 a
	Effect of extracts over growth rate of *Curvularia lunata* (lineal regression)			
Root	$y = 0.09x + 5.2$; r^2 0.97	$y = 0.16x + 3.3$; r^2 0.97	$y = 0.24x + 4.9$; r^2 0.96	$y = 0.09x + 5.2$; r^2 0.97
Stem	$y = 0.22x + 5.4$; r^2 0.97	$y = 0.03x + 4.5$; r^2 0.86	$y = 0.15x + 8.3$; r^2 0.86	$y = 0.22x + 5.4$; r^2 0.97
Leaf	$y = 0.28x + 9.1$; r^2 0.96	$y = 0.17x + 3.6$; r^2 0.97	$y = 0.14x + 4.7$; r^2 0.94	$y = 0.28x + 9.1$; r^2 0.96
Fruit	$y = 0.29x + 6.9$; r^2 0.94	$y = 0.09x + 4.1$; r^2 0.98	$y = 0.09x + 4.5$; r^2 0.94	$y = 0.29x + 6.9$; r^2 0.94
Control	$y = 0.30x + 5.8$; r^2 0.99			
Captan®	$y = 0.08x + 0.4$; r^2 0.93			
	Minimum inhibitory concentration (MIC) [µg· ml^{-1}] of methanolic extracts			
Root	112.50	112.50	225.00	225.00
Stem	112.50	112.50	112.50	112.50
Leaf	112.50	112.50	28.12	112.50
Fruit	112.50	56.25	112.50	450.00

Species	Activity of mixture extracts from *Solanum* spp.**	
	Tissues extracts mixture	Mycelial inhibition [%]
Solanum chrysotrichum	root, stem, leaf	64.9 ± 6.7 a
Solanum erianthum	root, steam, fruit	38.6 ± 4.0 bc
Solanum rostratum	root, stem, leaf, fruit	28.1 ± 15.8 c
Solanum torvum	root, steam, fruit	55.8 ± 21.3 ab
Interspecies mixture (IM)	fruit (*S. rostratum),* stem (*S. erianthum*), root (*S. chrysotrichum*, *S. torvum)*	41.5 ± 17.3 bc
	Conidia numbers*** [number · cm^{-2}]	
Solanum chrysotrichum	root	29.4 ± 14.9 c
Solanum erianthum	stem	4.0 ± 2.2 a
Solanum rostratum	stem	6.5 ± 4.5 ab
Solanum torvum	root	9.0 ± 4.8 ab
Mixture *S. chrysotrichum*	root, stem, leaf	4.4 ± 2.3 a
Mixture *S. erianthum*	root, steam, fruit	3.8 ± 1.7 a
Mixture *S. rostratum*	root, stem, leaf, fruit	3.7 ± 2.1 a
Mixture *S. torvum*	root, steam, fruit	3.1 ± 1.6 a
Interspecies mixture	IM	3.6 ± 2.5 a
Control		13.6 ± 5.2 b

*Kruskal-Wallis Test. *Sc*: H: 38.09; p: 1.07E-07. *Se*: H: 42.04; p: 1.63E-08. *St*: H: 34; p: 7.45E-07. *Sr*: H: 43.6; p: 7.65E-09
**Kruskal-Wallis Test. H: 32.1; p: 4.88 E-07
***Kruskal-Wallis Test. *Sc*: H: 20.43; p: 2.1 E-04
Values in a row followed by different letters are significantly different ($p < 0.05$, Mann-Whitney test)

mixtures decreased the number of conidia to half compared with the control treatment (Table 1; $p = 0.0001$).

Koduru *et al.* (2006) reported that the methanolic extracts from the leaves and fruits of *S. aculeastrum* inhibited *Aspergillus flavus* and *Fusarium oxysporum* at 5 mg · ml^{-1}. This is 30 times more than the reported concentration in this research. In some studies, *Solanum* plants were inactive. For example, the methanolic extracts of leaves, roots and stems of *S. torvum* did not inhibit *A. niger, A. flavus, Colletotrichum falcatum* and *F. moniliforme*, and other fungi (Bari *et al.* 2010), whereas in the present study they were some of the most powerful. In contrast to the study of Bari *et al.* (2010) who reported that the *Solanum* extracts

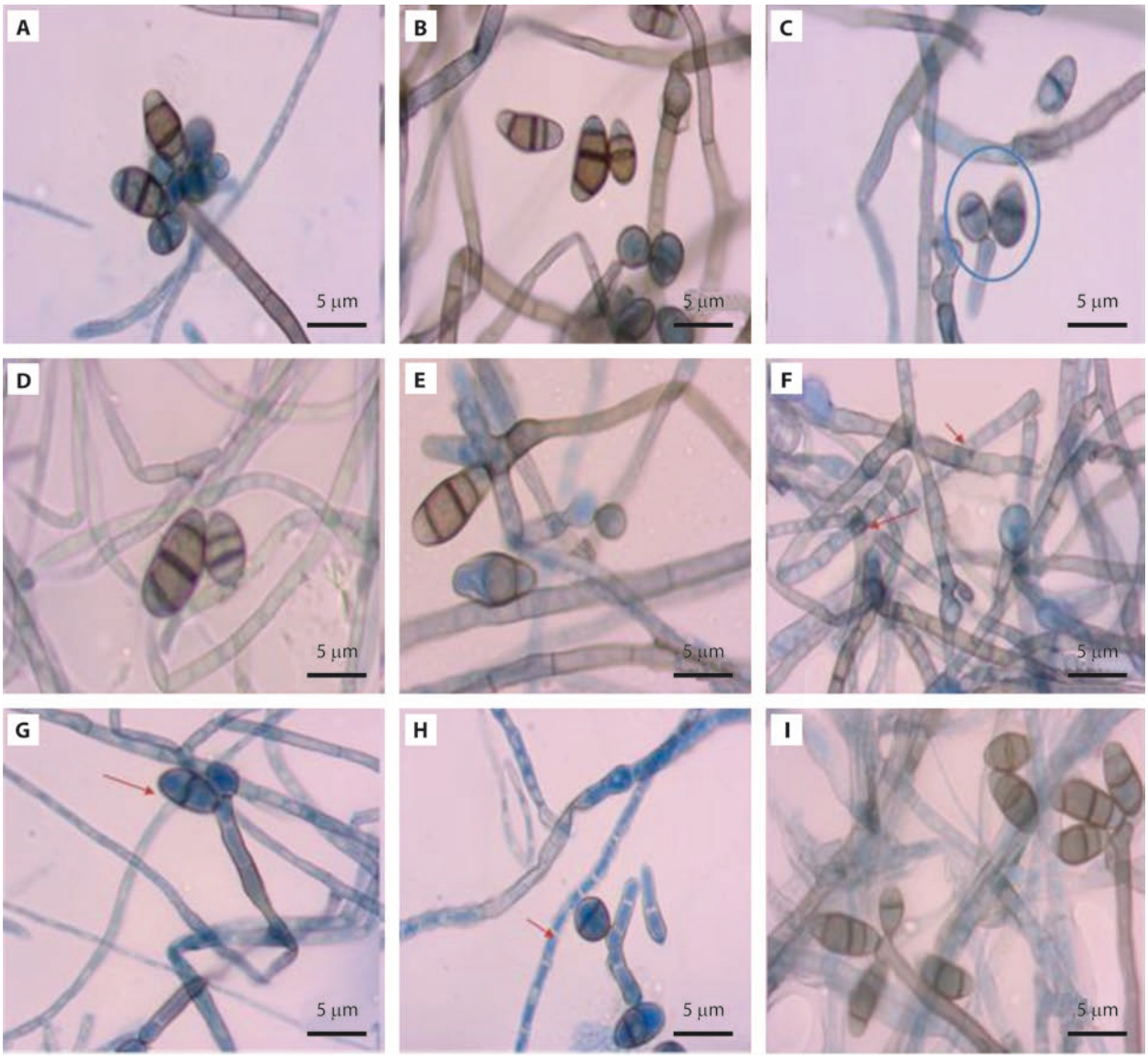

Fig. 1. Microscopic changes in morphology of *Curvularia lunata* provoked by *Solanum* spp. extracts. A – Sabourand Dextrose Agar (SDA); B – *S. chrysotrichum* (root); C – *S. torvum* (root); D – *S. erianthum* (stem); E and F – *S. rostratum* (fruit); G – *S. torvum* (*intra*-species mixture); H – *S. rostratum* (*intra*-species mixture); I – *inter*-species mixture

of stem exhibited no activity on phytopathogens, this study showed that the stem extracts of all species had a medium to high potency (MIC 112.8 µg · ml^{-1}).

The positive effect of mixtures on agar may indicate that the chemical profile of each tissue collaborated with the increase in total activity, perhaps because there is a greater number of active molecules that act independently (additive or synergistic effect), or because the extracts contain common active principles raising their proportion. Furthermore, some non-active molecules facilitate the absorption of active polar molecules through the membranes, increasing its bioavailability (Bassolé and Juliani 2012).

Each *Solanum* species showed differences in the inhibition capacity against *C. lunata*. This indicates that each species possesses different active components and that these metabolites vary in concentration. From the methanolic extracts of *S. chrysotrichum* leaves, saponins have been obtained which have antifungal activity (Herrera-Arellano *et al.* 2013). Bioactive steroidal alkaloids have been isolated from leaves of *S. torvum* and *S. chrysotrichum* (Pérez-Amador *et al.* 2007; Chou *et al.* 2012).

In conclusion, the decrement of the conidia production and mycelial inhibition caused by extracts of *Solanum* is relevant because it controls the reproduction and development of *C. lunata* and shows the potentiality of *Solanum* species to develop a potential fungicide based on their active components with antifungal activity.

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ZGH, CARS and IDC conducted experiments. IDC, ZGH, and MCM analyzed the data. IDC, ARGE and MCM wrote the article. IDC and ARGE contributed reagents and equipment. All authors read and approved the manuscript.

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