

In vitro antifungal activity of *Ricinus communis* and *Cyperus rotundus* on *Colletotrichum gloeosporioides* strains

Actividad antifúngica *in vitro* de *Ricinus communis* y *Cyperus rotundus* sobre cepas de *Colletotrichum gloeosporioides*

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RESUMEN

Antecedentes: *Colletotrichum gloeosporioides* genera antracnosis en diversos cultivos agrícolas.

Objetivo: Evaluar la actividad antifúngica de *Ricinus communis* y *Cyperus rotundus* sobre las cepas WSFT y Tainung de *Colletotrichum gloeosporioides*, y compararla con productos comerciales a base de *Bacillus subtilis* (Serenade®) y *Streptomyces* spp. (Blite Free®).

Métodos: Se determinaron los porcentajes de inhibición mediante un bioensayo de dosis-respuesta con Serenade® y Blite Free® y los extractos etanólicos de *R. communis* y *C. rotundus* sobre cepas de *C. gloeosporioides* aisladas de *Carica papaya*.

Resultados y conclusiones: *R. communis* indujo una mayor inhibición micelial (62.7 %) en la cepa Tainung comparado con Serenade® (54.2 %) y Blite Free® (0 %). Los valores de CE_{50} obtenidos en la cepa WSFT fueron similares entre *R. communis* (0.80 %) y Serenade® (0.79 %). La actividad antifúngica de los extractos vegetales resultó comparable a la de los productos comerciales, lo que sugiere su potencial para el control de *C. gloeosporioides*.

Palabras clave: Antracnosis, CE_{50} , fitopatógeno, inhibición micelial, malezas

ABSTRACT

Background: *Colletotrichum gloeosporioides* causes anthracnose in various agricultural crops.

Objective: To evaluate the antifungal activity of *Ricinus communis* and *Cyperus rotundus* against WSFT and Tainung strains of *C. gloeosporioides*, comparing them with commercial products containing *Bacillus subtilis* (Serenade®) and *Streptomyces* spp. (Blite Free®).

Methods: Inhibition percentages were determined through a dose-response bioassay with Serenade® and Blite Free® and the ethanolic extracts of *R. communis* and *C. rotundus* on the strains of *C. gloeosporioides* isolated from *Carica papaya*.

Results and conclusions: *R. communis* induced higher mycelial inhibition (62.7 %) in the Tainung strain compared to Serenade® (54.2 %) and Blite Free® (0 %). The EC_{50} values obtained in the WSFT strain were similar between *R. communis* (0.80 %) and Serenade® (0.79 %). The antifungal activity of the plant extracts proved comparable to that of commercial products, suggesting their potential for controlling *C. gloeosporioides*.

Keywords: Anthracnose, LD_{50} , mycelial inhibition, phytopathogen, weeds

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Colletotrichum gloeosporioides (Penz.) stands out as the primary phytopathogenic fungus within the *Colletotrichum* genus, causing anthracnose, a primary post-harvest disease affecting fruits and vegetables (Mudiyanselage *et al.* 2019). The predominant method for anthracnose management involves chemical fungicides; however, this approach has notable drawbacks, including the development of fungal resistance and concerns related to human health and environmental risks (Bussaman *et al.* 2012). Consequently, there is a growing interest in exploring effective and environmentally friendly alternatives, such as the utilization of plant extract and rhizosphere bacteria (Botanelli *et al.* 2019, Banaras *et al.* 2021, Sharf *et al.* 2021). In this context, the plant extracts from the noxious weeds coco-grass (*Cyperus rotundus* L.) and castor bean (*Ricinus communis* L.) inhibit the growth of fungi belonging to the genera *Fusarium*, *Alternaria*, *Curvularia*, and *Colletotrichum* (Hernández-Albíter *et al.* 2007, Singh *et al.* 2011, Luque *et al.* 2014, Mudiyanselage *et al.* 2019). As a result, these weed plants emerge valuable alternatives to chemical fungicides or even commercial bacterial-based products, given that plant extracts often exhibit a broad spectrum of antifungal activity due to their diverse array of phytochemicals, reducing the likelihood of fungal resistance (Ashwini and Srividya 2014, Kim *et al.* 2014, Ruiz-Sánchez *et al.* 2014, Landero-Valenzuela *et al.* 2016, Rashmi *et al.* 2019, Banaras *et al.* 2020, Javaid *et al.* 2020). However, the assessment of the effectiveness of these plants and bacterial-based products against the same phytopathogenic fungi has been limited. Building on the above, the objective of this study was to evaluate the *in vitro* antifungal activity of ethanolic extracts from *C. rotundus* and *R. communis* against *C. gloeosporioides*. This evaluation was performed in comparison with the commercial bacterial-*Bacillus subtilis* (Serenade®) and *Streptomyces* spp. (Blite Free®), with the intention of gauging the potential of these plant extracts relative to their commercial counterparts.

Leaves of *R. communis* and *C. rotundus* were collected from Tecoman, Colima, Mexico, and dehydrated (45-50 °C, two days) in a hot-air oven (Felisa®, Model FE-291A, Mexico), and the commercial products Serenade® (ASO, Bayer, Mexico) and Blite Free® (Altus Biopharm, Mexico) were purchased from a local retailer. The ethanolic extracts were obtained from

50 g of dried leaves of *R. communis* and *C. rotundus* plants (Bussaman *et al.* 2012). The WSFT and Tainung *Colletotrichum gloeosporioides* strains, source from the fungi collection unit at the School of Biology and Agricultural Science, Universidad de Colima. These strains were isolated from *Carica papaya* L. var. "Maradol", and cultured in potato dextrose agar medium (PDA, Bioxon®, Mexico) at 25 °C and 75 % relative humidity, for seven days. Culture media with different concentrations (0, 0.125, 0.25, 0.50 and 1.0 %, v/v) of the *R. communis* (EER) and *C. rotundus* (EEC) extracts and the commercial bacterial-based products were prepared (Bussaman *et al.* 2012). PDA served as a negative control. The dose-response bioassay involved inoculating 5 µL of the spore solution (1×10^7 conidia. mL⁻¹) at the centre of the plate. Five replications were made for each treatment. The percentage of inhibition of mycelial growth (% IMG) was calculating using the equation $\% \text{ IMG} = [(C - T) / C] \times 100$, where C represents the mycelium area in the Petri dish of the control group (without extracts and commercial bacteria-based products) and T, the mycelium area in the Petri dish with extract and commercial bacteria-based products (Zamora *et al.* 2008). The % IMG underwent one- and two- factor analysis of variance, each with five levels. Means were compared using Student's t-test with a Least Significant Difference (LSD) of 0.05. The effective concentration (EC₅₀) was determined by a Probit analysis using the Statistical Analysis System (SAS Institute Inc., 2002) only for treatments in which more than 50 % of IMG was observed.

In the WSFT strain, the highest mycelial inhibition (52.9 %) ($P \leq 0.046$) was observed with 1 % Serenade®, followed by the 0.5 % and 1 % of EER with inhibitions of 49 % and 48.7 % respectively. Regarding the Tainung strain, the most significant inhibition (62.7 %) ($P < 0.0001$) was achieved at 1 % EER, surpassing the mycelial inhibition observed at any Serenade® doses (Table 1). Only the EER and Serenade® induced a 50 % inhibition of mycelial growth in both strains. The EC₅₀ values obtained in the WSFT strain were similar between both products, although the confidence interval was wider with Serenade® ($P \leq 0.0015$) (Table 2). Concerning the Tainung strain, the EC₅₀ obtained with the EER was approximately three times greater (1.26 %) compared to Serenade® (0.43 %), the latter representing the lowest EC₅₀ value observed in this

study. Accordingly, EER and the commercial product demonstrated a similar efficacy in inhibiting the mycelial growth of WSFT strain, but the Serenade® exhibited greater inhibition in the Tainung strain.

Table 1. Mycelial inhibition growth (%) of two *C. gloeosporioides* strains in potato dextrose agar containing ethanolic plant extracts and commercial biological products

WSFT strain				
Doses (%)	EER	EEC	Serenade®	Blite Free®
0.125	0	0	42.1 ± 2.3 b	0
0.25	0	0	45.5 ± 2.3 ab	0
0.5	49.0 ± 3.4	0	44.5 ± 3.4 b	0
1	48.7 ± 2.2	30.1 ± 3.1	52.9 ± 1.5 a	0
<i>P</i>	DNSA	DNSA	0.0406	--
<i>F</i>	DNSA	DNSA	3.48	--
Tainung strain				
Doses (%)	EER	EEC	Serenade®	Blite Free®
0.125	18.2 ± 1.8 b	0	46.7 ± 1.3 b	0
0.25	7.3 ± 1.0 c	0	47.7 ± 1.8 b	0
0.5	5.6 ± 2.4 c	0	48.5 ± 2.4 b	0
1	62.7 ± 1.9 a	39.0 ± 1.4	54.2 ± 1.5 a	0
<i>P</i>	0.0001	DNSA	0.0504	--
<i>F</i>	231.22	DNSA	3.23	--

Means (±SE) with distinct letters are significantly different from each other (LSD, $P < 0.05$). *P*=*P*-value. *F*=Fisher value. EER: ethanolic extract of *R. communis*. EEC: ethanolic extract of *C. rotundus*. DNSA=data did not support the analysis.

The greater inhibition (62 %) observed in present research was lower compared to a previous report (73 %) from the essential oil of *R. communis* (Siqueira-Júnior *et al.* 2012). However, it surpassed the inhibition percentage obtained in *C. gloeosporioides* (19 %) (Mudiyanselage *et al.* 2019), and *Colletotrichum musae* (0 %) (Bazie *et al.* 2014), both from methanolic extract of leaves of the same plant species. The differences in inhibition values in these studies may be attributed, in part, to the compounds obtained in each solvent used in the extraction process. The polar index of methanol is higher than those of ethanol, therefore, the latter extracts chemical compounds with slightly higher hydrophobicity compared to compounds extracted using methanol (Abarca-Vargas *et al.* 2016). Concerning *C. rotundus*, Luque *et al.* (2014) reported a mycelial inhibition of *C. gloeosporioides* of 61 % and 74 % from aqueous extracts of corms and leaves of *C. rotundus*, respectively. These values are higher than the inhibition obtained (39 %) using an ethanolic extract in this research but lower than those observed with *R. communis*, which may be attributed to the compounds produced by each plant species, and the compounds extracted in the solvents. On the other hand, Ashwini and Srividya (2014) reported the inhi-

bition of *B. subtilis* (57 %) against *C. gloeosporioides*, which was slightly greater than the 54 % obtained with the *Bacillus*-based commercial product used in our research, but lower than the 62 % observed with the EER. Likewise, Ruiz-Sánchez *et al.* (2014) reported an inhibition range between 63 % and 80 % of some *B. subtilis* strains against *C. gloeosporioides* by the *in vitro* direct confrontation method, and from 13 % to 42 % in the *in vitro* bacterial filtration assay. The difference in the inhibition values may be due to bioassay performed in each study to evaluate the antifungal activity of *Bacillus* strains. Besides, the strains A1022 and A1022SC of *Streptomyces* induced inhibitions of 22 % and 56 %, respectively, in a disk diffusion assay against *C. gloeosporioides* (Lee *et al.* 2011). These values surpass those observed with Blite Free® in the present study but are lower than those obtained with the EER (62 %) and the EEC (39 %) in the Tainung strain. In conclusion, the analysed weed plant species analysed have the potential as environmentally friendly fungicides against *C. gloeosporioides*, given their abundant presence in agricultural fields. Future studies are needed to identify the key components responsible for the antifungal activities of the plant extracts.

Table 2. Mean effective concentration (EC₅₀) of ethanolic plant extracts and commercial biological products on two *C. gloeosporioides* strains

WSFT strain						
Treatment	EC ₅₀ (%)	CI (%)	Slope	Equation Probit	X ²	P>Chi-X ²
EER	0.80	0.75-0.86	2.91	Y=2.91X+0.27	404.8	0.0001
Serenade®	0.79	0.48-3.57	0.26	Y=0.26X+0.03	10.09	0.0015
Tainung strain						
Treatment	EC ₅₀ (%)	CI (%)	Slope	Equation Probit	X ²	P>Chi-X ²
EER	1.26	1.08-1.54	1.44	Y=1.44X-0.15	220.05	0.0001
Serenade®	0.43	0.18-2.7	0.19	Y=0.19X+0.07	5.46	0.0195

CI=confidence interval. EER=ethanolic extract of *R. communis*.

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