# Essential Oils and Extracts from Argentinian Northwest Plants as Potential Biofungicides for Olive and Grapevine Pathogens: *in vitro* Studies

## Aceites esenciales y extractos de plantas del noroeste argentino como potenciales biofungicidas de patógenos de olivo y vid: estudios *in vitro*

María Sayago [1, 2], Ivana Ormeño [2], María Teresa Ajmat [2], Natalia Barbieri [1, 2*]

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**Abstract**

This work studies the effect of 12 botanical products from Argentinian northwest plants on spores and mycelium of *Verticillium dahliae* and *Phaeoacremonium parasiticum*, two pathogens of agronomic importance for the region. The fungi were exposed to essential oils (EOs) or ethanolic extracts (EEs), determining the percentage of germinated spores and mycelial growth. All tested EOs and EEs showed varying degrees of antifungal activity, dependent on plant species, extract type, pathogen, and targeted fungal structures. *V. dahliae* germination was completely inhibited by *Zuccagnia punctata* and *Clinopodium gilliesii* EOs. In experiments with EEs, *Z. punctata* EE was the most effective in suppressing spore germination of both fungi. The *C. gilliesii* EE also controlled *V. dahliae* germination. The EEs of *Z. punctata, C. gilliesii* and *Lippia turbinata* were the most active against mycelial growth. These three EEs had a fungistatic effect on *P. parasiticum* while *Z. punctata* and *L. turbinata* EEs showed a fungicidal effect on *V. dahliae*. The products obtained from *Z. punctata*, *C. gilliesii* and *L. turbinata* have potential as biocontrollers against *V. dahliae* and *P. parasiticum.* This is encouraging since no effective treatments are available for the diseases involving these pathogens.

**Keywords**
*Verticillium dahliae* Kleb • *Phaeoacremonium parasiticum* (Ajello, Georg & C. J. K. Wang) W. Gams, Crous & M. J. Wingf • botanical antifungals • mycelial inhibition • conidial susceptibility

1 Universidad Nacional de Chilecito. CONICET. Departamento de Ciencias Básicas y Tecnológicas. 9 de Julio 22, Chilecito F5360CKB. La Rioja. Argentina.
* nbarbieri@undec.edu.ar
2 Universidad Nacional de Chilecito. Instituto de Ambiente de Montaña y Regiones Áridas.

**Resumen**

Este trabajo estudia el efecto de 12 productos de plantas del noroeste argentino sobre las esporas y micelio de *Verticillium dahliae* y *Phaeoacremonium parasiticum*, dos patógenos de importancia agronómica. Los hongos fueron expuestos a los aceites esenciales (AE) o extractos etanólicos (EE), y se determinó el porcentaje de germinación y crecimiento micelial. Todos los AE y EE mostraron actividad antifúngica, la cual dependió de la especie vegetal, del extracto, del patógeno y de las estructuras fúngicas objetivo. La germinación de *V. dahliae* fue inhibida con los AE de *Zuccagnia punctata* y *Clinopodium gilliesii*. El EE de *Z. punctata* fue el más efectivo para suprimir la germinación de ambos hongos. El EE de *C. gilliesii* también fue capaz de controlar la germinación de *V. dahliae*. Mientras que los EE de *Z. punctata*, *C. gilliesii* y *Lippia turbinata* fueron los más activos sobre el micelio. Estos tres EE fueron fungistáticos sobre *P. parasiticum* mientras que los EE de *Z. punctata* y *L. turbinata* fueron fungicidas sobre *V. dahliae*. Los productos obtenidos de *Z. punctata, C. gilliesii* y *L. turbinata* son potenciales biocontroladores de *V. dahliae* y *P. parasiticum*. Esto es alentador ya que no se dispone de tratamientos eficaces para las enfermedades en las cuales participan estos patógenos.

**Palabras clave**

*Verticillium dahliae* Kleb • *Phaeoacremonium parasiticum* (Ajello, Georg & C. J. K. Wang) W. Gams, Crous & M. J. Wingf • antifúngicos botánicos • inhibición micelial • susceptibilidad conidial

## Introduction

Olive and grapevine cultivation in La Rioja province (northwest Argentina) is economically significant. Fungal diseases affect productivity causing considerable losses (7, 12). Vascular wilt disease in olives caused by *Verticillium dahliae* Kleb has acquired great importance worldwide producing tree mortality, fruit yield reduction, and organoleptic defects in virgin olive oil extracted from infected plants (18, 19). Olive verticillium wilt is one major concern for olive growers in the semi-arid regions of Argentina. Rattalino (2023) has recently shown that *V. dahliae* is widely spread in La Rioja olive-growing regions, estimating 24% disease incidence.

Grapevine trunk diseases are the principal fungal diseases affecting viticulture worldwide (17). Among these pathologies, *hoja de malvón* (related to Esca) and young vine decline (Petri disease) are among the most devastating and challenging diseases in many wine regions of Argentina. They are caused by multiple wood fungal pathogens, with *Phaeoacremonium parasiticum* being mostly prevalent (9, 10).

Unfortunately, effective treatments against these mycoses are not available, and their management remains difficult. To date, recommendations focus on timely monitoring of these diseases and integrated management strategies including biological control as a potential tool (17, 19).

Plant essential oils (EOs), extracts and related molecules have demonstrated inhibitory efficacy against pathogenic fungi (3, 26). They represent eco-friendly control alternatives for integrated disease management, contributing to sustainable agricultural production. The antifungal activity (AA) of some EOs and a few plant extracts is reported against *V. dahliae* (6, 8, 11, 14, 24). However, insufficient studies focus on biological control of *P. parasiticum* using plant products. This study focused on plant species with previous AA against dermatophytes or molds: *Zuccagnia punctata, Clinopodium gilliesii, Lippia turbinata, Lippia integrifolia, Argemone subfusiformis, Erythrostemon gilliesii*, and *Senecio subulatus* var. *salsus* (1). We explore their AA against olive and grapevine pathogenic fungi, hypothesizing that plant products from these species could control plant pathogenic fungi in regional crops. We evaluated the effect of 12 botanical products (secondary metabolites) obtained from the mentioned plants on spore viability and mycelial growth of *V. dahliae* and *P. parasiticum*.

## Material and methods

### Plant Material

*Z. punctata* Cav., *C. gilliesii* Kuntze, *L. turbinata* Griseb., *L. integrifolia* Hieron., *A. subfusiformis* Ownbey., *E. gilliesii* (Hook.) Klotzsch and *S. subulatus var. salsus* (Griseb.) were collected in 2018. Georeferenced specimens were deposited in the herbarium of the Universidad Nacional de Chilecito (UNDEC). Supplementary Table 1 provides data on collection sites, yield and voucher specimens.

### Obtaining Essential Oils (EOs) and Ethanolic Extracts (EEs)

Air-dried canopies were used. EOs were obtained by hydrodistillation in a Clevenger-type apparatus and stored at -20°C until further use. To obtain EEs, the plant material was macerated in ethanol 96° for 24 h, filtered and the solvent evaporated. Then, waxes were removed by precipitation from an ethanol-water solution. Later, EEs were dissolved in 50% ethanol, shaken using a 40 kHz ultrasonic cleaning bath (1 h) and centrifuged (5000 rpm, 10 min). Finally, the separated supernatants were evaporated and samples were stored until use (2).

### Phytopathogenic Fungi

We used a native non-defoliating strain of *V. dahliae* Kleb. previously isolated from an infected olive plant in La Rioja (21). The *P. parasiticum* strain was obtained from the Phytopathology Laboratory of INTA Mendoza, Argentina. First, stock cultures (stored at -80°C) were activated in potato dextrose agar (PDA, Britania, Argentina) and grown in microcultures (PDA block on a microscopic slide) to check morphological traits (Supplementary Figure 1). Secondly, fungi were maintained in PDA for antifungal assays.

### Inhibition of Spore Germination

The phytopathogens *V. dahliae* and *P. parasiticum* were cultured for 7 and 14 days, respectively, allowing spore development. To obtain spores, 2 mL sterile distilled water were added, and mycelia was gently scraped with a Drigalsky spatula. The suspension was recovered and adjusted to 1 x $10^3$ spores/mL using a Neubauer counting chamber. For the assays, a 100 uL spore suspension was incubated with 100 uL of different concentrations of EO or EE (1-3 mg/mL) for 1h at 24°C. For plant products with 100% inhibitory activity at 1 mg/mL, lower concentrations (range of 0.2-1 mg/mL) were also evaluated. Following incubation, an aliquot was taken and seeded in PDA. After 48 h for *V. dahliae* and 72 h for *P. parasiticum* at 24°C incubation, spores were counted and the percentage of inhibited spores (number of non-germinated spores/total number of spores × 100) was determined (16). Growth control for each tested phytopathogen (distilled water), solvent control (DMSO or ethanol 96°) and EO and EE sterility controls were included. According to own experimental data, Benomyl (fungicide) constituted the positive control in concentrations ranging from 0.1 to 0.4 mg/mL for *V. dahliae* and 7 to 10 mg/mL for *P. parasiticum*. The minimum inhibitory concentration (MIC) was defined as the lowest EO or EE concentration producing 100% inhibition of spore germination.

### Synergism (Checkerboard Test)

The MIC values obtained previously served as a reference and combinations ranging from 0.125xMIC to 1xMIC of EOs, EEs and Benomyl were formulated. Inhibition on spore germination was determined using the methodology described above. To evaluate combination effects, the fractional inhibitory concentration (FIC) index was calculated as FIC index = FICA + FICB, where FICA and FICB are the minimum concentrations inhibiting fungal growth (MIC) for samples A and B, respectively. FICA = (Combination MICA) / (MICA alone), FICB = (Combination MICB)/ (MICB alone). According to the FIC index, results indicated synergism (≤ 0.5), addition (> 0.5 and ≤ 1.0), indifference (> 1.0 and ≤ 2.0), or antagonism (> 2.0) (25).

**Inhibition of Mycelial Growth**

EEs were added at different concentrations (0.25-3 mg/mL) on molten PDA. Petri dishes with PDA plus EE were inoculated with a 5-mm diameter mycelial disc obtained from the edge of 7-and 14-day-old cultures of *V. dahliae* and *P. parasiticum*, respectively. Growth control for each tested phytopathogen (PDA plate with the mycelial disc), solvent control (PDA plate plus 96° ethanol with the mycelial disc), and positive control (PDA plate plus Benomyl with the mycelial disc) were included. Inoculated plates were incubated at 24°C and growth of *V. dahliae* and *P. parasiticum* was evaluated at 7 days by measuring mycelial diameter of each colony. Percentage of growth inhibition was calculated by equation 1:

$$\% \text{ inhibition} = ((D-d)/D) \times 100) \quad (1)$$

where
D = colony diameter of growth controls
d = diameter in EE or Benomyl treatments

The MIC was equal to the lowest EE concentration at which mycelial growth was completely inhibited (24). When EEs inhibitory effects were fungicidal or fungistatic, PDA plates with mycelium discs and different EE treatments would be incubated for 2-5 additional days (mycelial inhibition at 9 days for *V. dahliae* and 12 days for *P. parasiticum*). When no mycelium re-growth occurred during additional incubation, EE was considered fungicidal. Otherwise, it was considered fungistatic.

**Total Phenolic and Flavonoid Content (PC and FC) in the EEs**

Total PC was determined by the Folin-Ciocalteu spectrophotometric method. Different volumes of EE solutions were mixed with Folin-Ciocalteu reagent and sodium carbonate. After incubation, absorbance was measured at 765 nm. The PC was determined using a Gallic acid calibration curve and results were expressed as mg Gallic acid equivalents/g of dry extract (mg GAE/g) (2). FC was estimated by a spectrophotometric assay based on aluminum chloride complexes. Serial dilutions from EEs were mixed with aluminum chloride, and incubated for 1 h. Absorbance was measured at 420 nm. FC was calculated using a Quercetin calibration curve and expressed as mg quercetin equivalents/g of dry extract (mg QE/g) (2).

**Statistical Analysis**

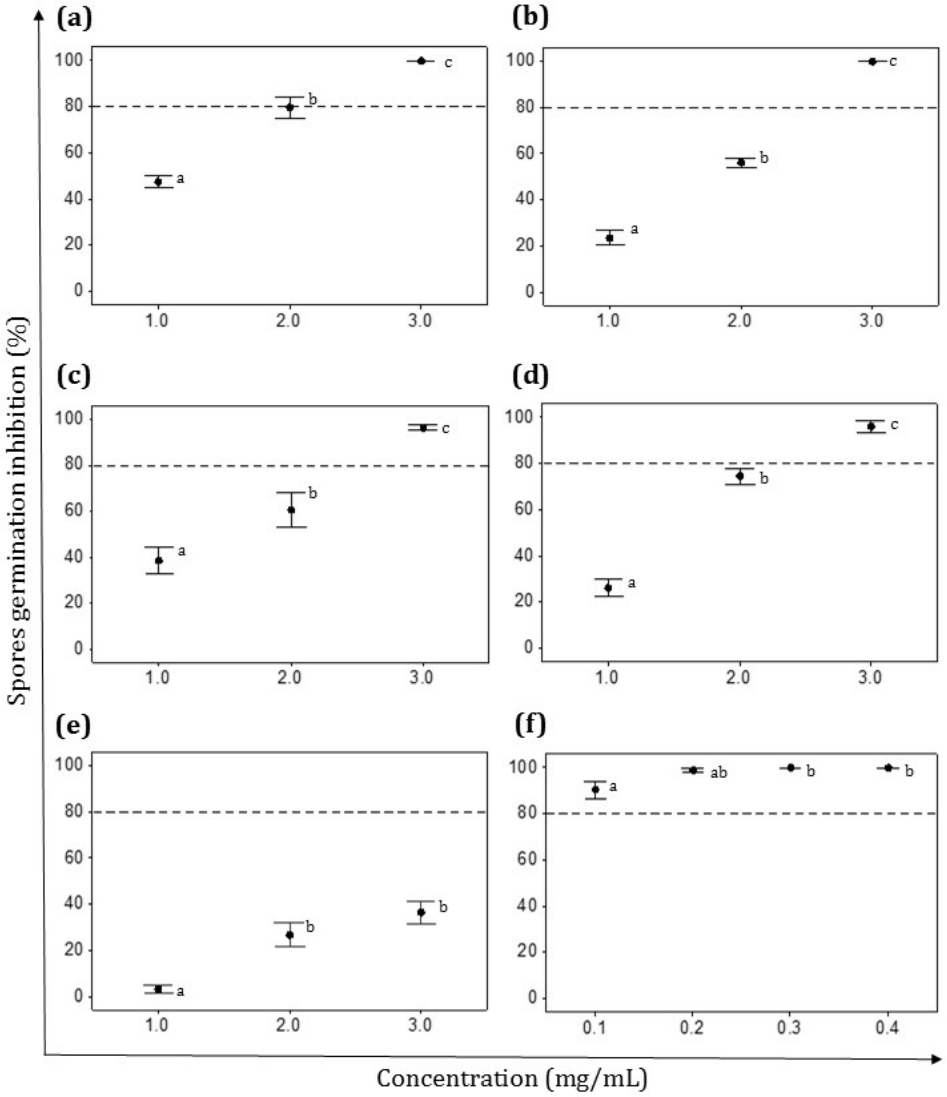
Each treatment had two replicates, and experiments were conducted at least three times using a randomized design. Results are expressed as mean ± standard deviation/standard error. Statistical significance of the data was determined by ANOVA followed by Tukey's test (MINITAB software version 15 for Windows, SPSS Inc., Chicago, IL), $p \geq 0.05$. Pearson's correlation coefficient was calculated between AA and phenols or flavonoid content of EEs using InfoStat software (5).

## Results

**Effect of EOs and EEs on *V. dahliae* and *P. parasiticum* Spore Germination**

The AA of five EOs and seven EEs obtained from plants in northwest Argentina was evaluated against spore germination of *V. dahliae* and *P. parasiticum*. Inhibition of conidia germination varied among treatments and increased with increasing EO or EE concentration.

Only the EOs from *Z. punctata* and *C. gilliesii* exhibited 100% inhibitory activity on *V. dahliae* spores, with MIC values of 3 mg/mL each (figures 1a and b, page 106). At the highest concentration tested, the EOs from *L. turbinata* and *L. integrifolia* showed remarkable activity against *V. dahliae* spores, with inhibition values of 96.4 and 96% respectively (figure 1c and d, page 106).




(a) *Z. punctata*, (b) *C. gilliesii*, (c) *L. turbinata*, (d) *L. integrifolia*, (e) *S. subulatus* var. *salsus*, (f) Benomyl (Fungicide).

Data are expressed as mean ± standard error, n=6-8. Different letters indicate significant differences among concentrations of the same EO. (p< 0.05).

(a) *Z. punctata*, (b) *C. gilliesii*, (c) *L. turbinata*, (d) *L. integrifolia*, (e) *S. subulatus* var. *salsus*, (f) Benomil (fungicida).

Los datos se expresan como la media ± error estándar, n=6-8. Letras distintas indican diferencias significativas entre concentraciones del mismo AE. (p< 0,05).

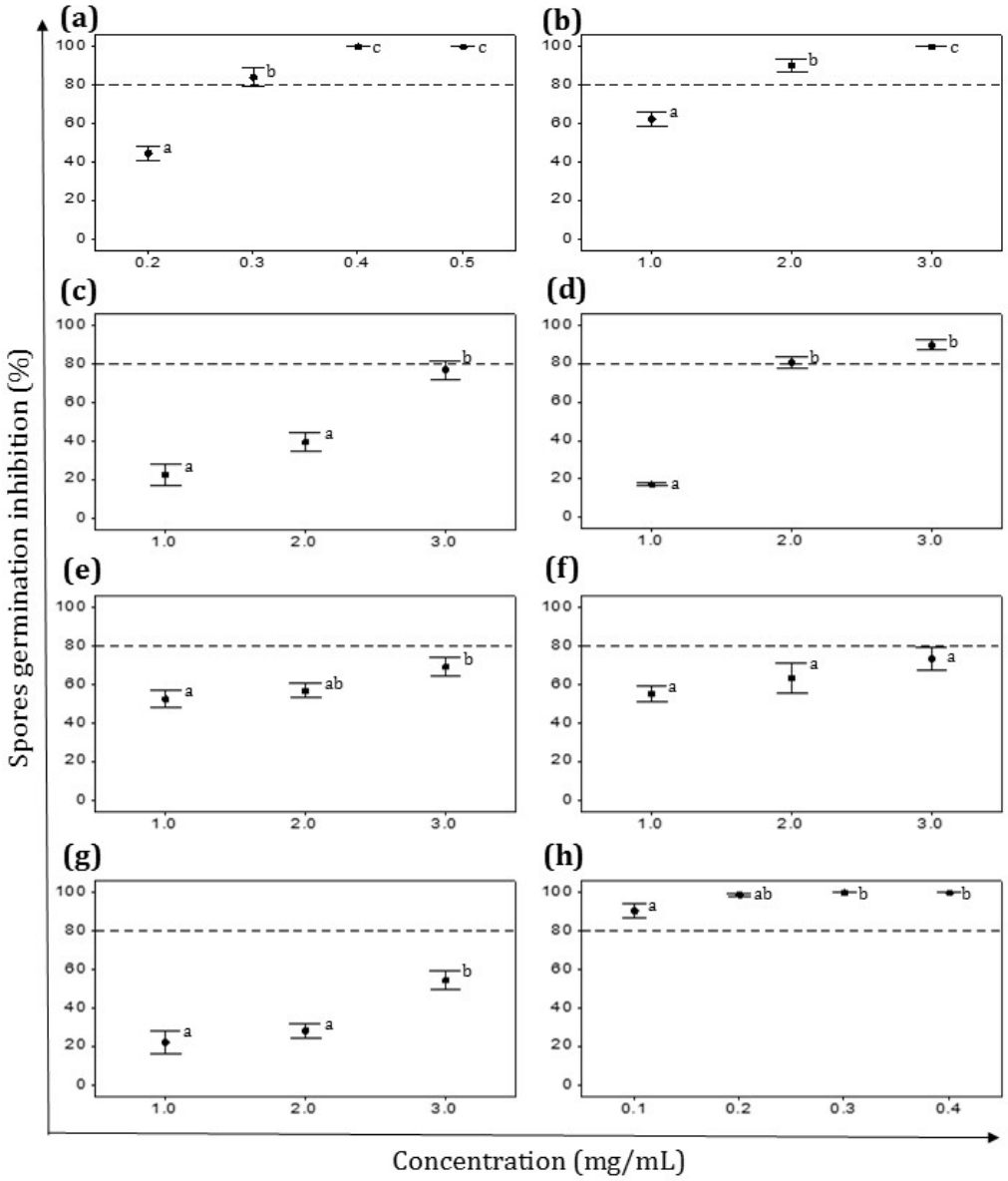
**Figure 1.** Effect of essential oils (EOs) on *V. dahliae* spore germination.

**Figura 1.** Efecto de los aceites esenciales (AE) sobre la germinación de las esporas de *V. dahliae*.

Concerning spore germination of *P. parasiticum*, no EO had a 100% inhibitory effect. *C. gilliesii* EO inhibited 85.8% of spores at 3 mg/mL, while the remaining oils showed low activity, with 30-54 % inhibition at the highest concentration evaluated (Supplementary Figure 2).

On the other hand, in assays with EEs, only *Z. punctata* EE effectively controlled spore germination of both pathogenic fungi (figure 2a and 3a, page 107). The effective concentration (MIC) of this extract on *V. dahliae* was 0.4 mg/mL, similar to the MIC obtained with the synthetic antifungal Benomyl (MIC=0.3 mg/mL) (figure 2a and h, page 107).

Spore germination of *V. dahliae* was also completely inhibited at 3 mg/ml of *C. gilliesii* EE (MIC), while other EEs showed inhibitions ranging between 54% and 89% (figure 2, page 107). *P. parasiticum* spore germination was controlled at 0.75 mg/mL of *Z. punctata* EE (MIC), a much lower value than the obtained with the antifungal Benomyl (MIC=10 mg/mL) (figure 3a and h, page 108). In addition, significant inhibition of *P. parasiticum* spore germination (94%) was obtained at 3 mg/mL of *E. gilliesii* EE (figure 3f, page 108).

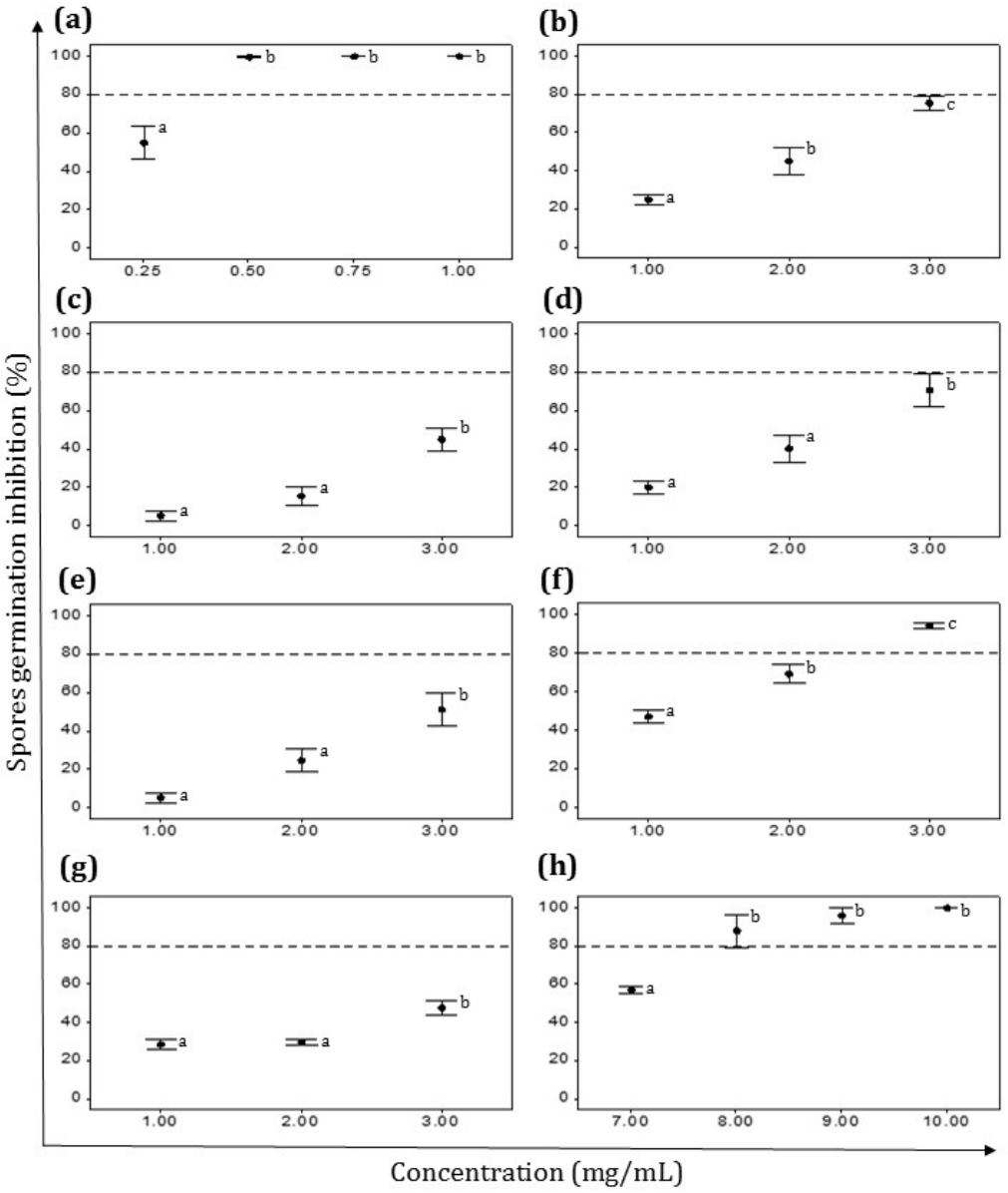



(a) *Z. punctata*, (b) *C. gilliesii*, (c) *L. turbinata*, (d) *L. integrifolia*, (e) *A. subfusiformis*, *(f) E. gilliesii*, (g) *S. subulatus* var. *salsus*, (h) Benomyl (Fungicide). Data are expressed as mean ± standard error, n=6-8. Different letters indicate significant differences among concentrations of the same EE. (p< 0.05).

(a) *Z. punctata*, (b) *C. gilliesii*, (c) *L. turbinata*, (d) *L. integrifolia*, (e) *A. subfusiformis*, (f) *E. gilliesii*, (g) *S. subulatus* var. *salsus*, (h) Benomyl (Fungicida). Los datos se expresan como la media ± error estándar, n=6-8. Letras distintas indican diferencias significativas entre concentraciones del mismo EE. (p< 0,05).

**Figure 2**. Effect of ethanolic extracts (EEs) on *V. dahliae* spore germination.

**Figura 2.** Efecto de los extractos etanólicos (EEs) sobre la germinación de esporas de *V. dahliae*.




(a) *Z. punctata*, (b) *C. gilliesii*, (c) *L. turbinata*, (d) *L. integrifolia*, (e) *A. subfusiformis*, (f) *E. gilliesii*, (g) *S. subulatus* var. *salsus*, (h) Benomyl (Fungicide). Data are expressed as mean ± standard error, n=6-8. Different letters indicate significant differences among concentrations of the same EE. ($p < 0.05$).

(a) *Z. punctata*, (b) *C. gilliesii*, (c) *L. turbinata*, (d) *L. integrifolia*, (e) *A. subfusiformis*, (f) *E. gilliesii*, (g) *S. subulatus* var. *salsus*, (h) Benomyl (Fungicida). Los datos se expresan como la media ± error estándar, n=6-8. Letras distintas indican diferencias significativas entre concentraciones del mismo EE. ($p < 0,05$).

**Figure 3.** Effect of ethanolic extracts (EEs) on *P. parasiticum* spore germination.

**Figura 3**. Efecto de los extractos etanólicos (EEs) sobre la germinación de esporas de *P. parasiticum*.

**Evaluation of Synergistic Antifungal Effect**

The results demonstrated no synergistic effect against *V. dahliae* and *P. parasiticum* spores for any of the evaluated combinations. Antifungal interaction was additive or indifferent (Supplementary Table 2).

**Effect of EEs on Mycelial Growth of *V. dahliae* and *P. parasiticum***

Since no EOs could completely inhibit *P. parasiticum* spore germination, and their activity on *V. dahliae* spore germination was weaker than the extracts, assays considering mycelial growth inhibition were performed with EEs only.

Mycelial growth inhibition increased with EEs concentration. All seven EEs tested showed growth inhibition of over 25% for both phytopathogens (figure 4, page 110 and figure 5, page 111).

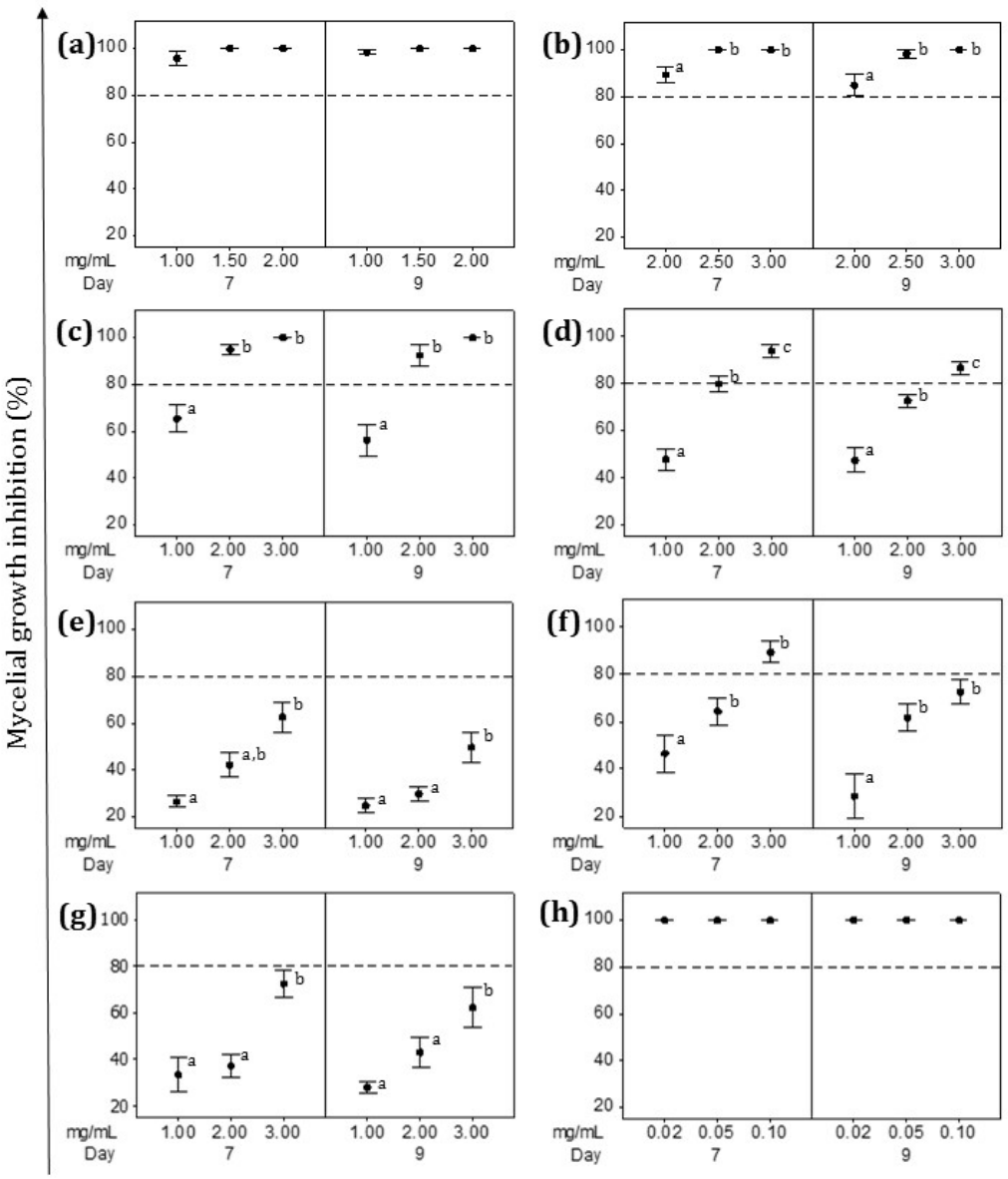
Considering EEs inhibitory effect on *V. dahliae*, three treatments (EEs from *Z. punctata, C. gilliesii* and *L. turbinata*) completely inhibited mycelial growth (figure 4a-c, page 110). *Z. punctata* EE was the most effective, obtaining the lowest MIC value (MIC=1.5 mg/mL for *Z. punctata* EE, MIC=2.5 mg/mL for *C. gilliesii* EE and MIC=3 mg/mL for *L. turbinata* EE; (figure 4a-c, page 110). The EEs of *L. integrifolia* and *E. gilliesii* reached inhibition values of 93.7% and 89.5% against *V. dahliae* at 3 mg/mL (figure 4d and f, page 110). The two remaining EE treatments (*A. subfusiformis* and *S. subulatus*) achieved 60-70% inhibition (figure 4e and g, page 110). Given that no mycelium re-growth occurred during additional incubation time (day 9), *Z. punctata, L. turbinata, L. integrifolia, A. subfusiformis* and *S. subulatus* EEs resulted fungicidal against *V. dahliae* (figure 4, page 110). In the case of *C. gilliesii* and *E. gilliesii*, mycelial recovery was observed at 9 days. The *C. gilliesii* EE MIC value changed from 2.5 to 3 mg/mL while inhibition percentage of *E. gilliesii* EE at 3 mg/mL decreased significantly (figure 4b and f, page 110). Thus, AA of these EEs on *V. dahliae* was considered fungistatic.

*P. parasiticum* mycelial growth was completely inhibited by *Z. punctata* and *L. turbinata* EEs (MIC=1 mg/mL for *Z. punctata* EE and MIC=2 mg/mL for *L. turbinata* EE) (figure 5a and c, page 111). In addition, the EEs of *C. gilliesii, L. integrifolia,* and *E. gilliesii* showed strong inhibitory effects on *P. parasiticum* mycelial growth reaching 92.6, 81.4 and 84.9% at 3 mg/mL, respectively (figure 5b, d and f, page 111). The EE treatments *A. subfusiformis* and *S. subulatus* also inhibited 60-70% of mycelial growth (figure 5e and g, page 111). The EE treatments produced reversible inhibition of *P. parasiticum* mycelium growth. During additional incubation time, the MIC value of *Z. punctata* and *L. turbinata* EEs increased to 2 mg/mL and 3 mg/mL, respectively. A significant reduction in inhibition percentage of the other EEs was also observed on day 12 (figure 5, page 111). Therefore, these EEs were fungistatic against *P. parasiticum*.

**Phenolic and Flavonoid Contents (PC and FC) in EEs**

Both PC and FC of the studied EEs were significantly different (Supplementary Table 3). The EE of *Z. punctata* showed the highest PC, followed by *C. gilliesii* EE, *L. turbinata* EE, *L. integrifolia* EE = *A. subfusiformis* EE = *E. gilliesii* EE, and *S. subulatus* EE. Regarding FC, *Z. punctata* EE presented the highest value (327.6 mg QE/g) and the other EEs ranged between 13 and 73 mg QE/g.

Pearson's correlation coefficient between PC and spore germination inhibition was r=0.63 ($p < 0.0001$) for *V. dahliae* and r=0.39 ($p < 0.0001$) for *P. parasiticum.* Additionally, a significant correlation was observed between PC and mycelial growth inhibition, with values of r=0.79 ($p < 0.0001$) for *V. dahliae* and r=0.78 ($p < 0.0001$) for *P. parasiticum*. The FC and inhibition of spore germination showed correlation coefficients of r=0.73 ($p < 0.0001$) for *V. dahliae* and r=0.72 ($p < 0.0001$) for *P. parasiticum,* while for FC and mycelial growth inhibition, r=0.60 ($p < 0.0001$) was observed for *V. dahliae* and r=0.67 ($p < 0.0001$) for *P. parasiticum*.

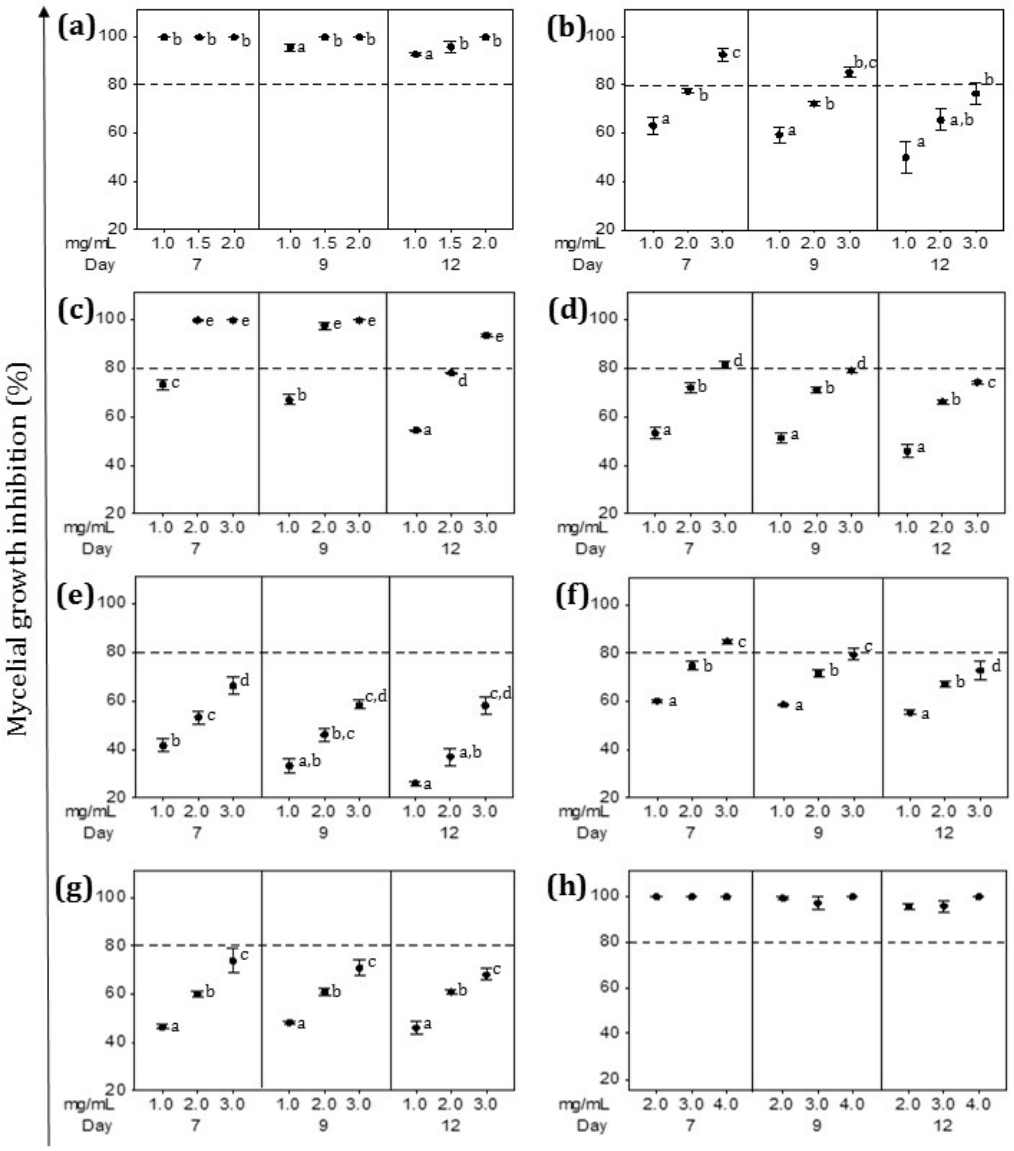



(a) *Z. punctata*, (b) *C. gilliesii*, (c) *L. turbinata*, (d) *L. integrifolia*, (e) *A. subfusiformis*, (f) *E. gilliesii*, (g) *S. subulatus* var. *salsus*, (h) Benomyl (Fungicide). The percentage of inhibition was determined at 7 and 9 days of incubation at 24°C. Data are expressed as mean ± standard error, n=6-8. Different letters indicate significant differences among the concentrations tested (p< 0.05).

(a) *Z. punctata*, (b) *C. gilliesii*, (c) *L. turbinata*, (d) *L. integrifolia*, (e) *A. subfusiformis*, (f) *E. gilliesii*, (g) *S. subulatus* var. *salsus*, (h) Benomyl (Fungicida). El porcentaje de inhibición se determinó a los 7 y 9 días de incubación a 24°C. Los datos se expresan como media ± error estándar, n=6-8. Letras diferentes indican diferencias significativas entre las concentraciones ensayadas. (p< 0,05).

**Figure 4**. Effect of ethanolic extracts (EEs) on *V. dahliae* mycelial growth.

**Figura 4**. Efecto de los extractos etanólicos (EE) sobre el crecimiento micelial de *V. dahliae*.




(a) *Z. punctata*, (b) *C. gilliesii*, (c) *L. turbinata*, (d) *L. integrifolia*, (e) *A. subfusiformis*, (f) *E. gilliesii*, (g) *S. subulatus* var. *salsus*, (h) Benomyl (Fungicide). The percentage of inhibition was determined at 7, 9 and 12 days of incubation at 24°C. Data are expressed as mean ± standard error, n=6-8. Different letters indicate significant differences among concentrations. (p< 0.05).

(a) *Z. punctata*, (b) *C. gilliesii*, (c) *L. turbinata*, (d) *L. integrifolia*, (e) *A. subfusiformis*, (f) *E. gilliesii*, (g) *S. subulatus* var. *salsus*, (h) Benomyl (Fungicida). El porcentaje de inhibición se determinó a los 7, 9 y 12 días de incubación a 24°C. Los datos se expresan como media ± error estándar, n=6-8. Letras diferentes indican diferencias significativas entre concentraciones. (p< 0,05).

**Figure 5**. Effect of ethanolic extracts (EEs) on *P. parasiticum* mycelial growth.

**Figura 5**. Efecto de los extractos etanólicos (EE) sobre el crecimiento micelial de *P. parasiticum*.

## Discussion

*V. dahliae* and *P. parasiticum* are important phytopathogens in La Rioja province, involved in Verticillium wilt of olive and grapevine trunk diseases, respectively (10, 20). Given the lack of control treatments, searching for antifungal agents is strategic (17, 19). We tested EOs and EEs from seven Argentinian northwest plants as natural alternatives against *V. dahliae* and *P. parasiticum*.

Three mg/mL of our EOs had remarkable activity against *V. dahliae* spore germination (100% inhibitory activity for *Z. punctata* and *C. gilliesii* EOs, and 96% inhibitory activity for *L. turbinata* and *L. integrifolia* EOs). Similarly, other EOs (orégano, thyme, laurel, and lavender) block *V. dahliae* conidia germination at concentrations ranging from 0.2 to 3 mg/mL (11, 14).

On the other hand, only the *C. gilliesii* EO was able to significantly inhibit *P. parasiticum* conidia germination, suggesting *V. dahliae* spores are more susceptible to the tested EOs than *P. parasiticum*. In addition, although previous reports demonstrated the activity of *C. gilliesii* and *L. turbinata* EOs against other phytopathogenic fungi (15, 22, 27), this is the first report on AA of *Z. punctata* and *L. integrifolia* EOs against this type of pathogens.

Based on EEs activity on conidia germination, only *Z. punctata* EE was able to control both *V. dahliae* (MIC= 0.4 mg/mL) and *P. parasiticum* (MIC=0.75 mg/mL). These results coincide with previous research showing the *Z. punctata* EE effectiveness against soybean pathogenic and brown rot fungi spore development at concentrations between 0.25-0.5 mg/mL (4, 23). Our results also showed that *C. gilliesii* EE controlled germination of *V. dahliae* spores, apparently never studied before against phytopathogenic fungi.

Although no synergistic antifungal effect was found for the mixtures of EO and EE tested, antagonistic absence and additive effects of the combinations of *Z. punctata* EO/*Z. punctata* EE and *C. gilliesii* EO/*Z. punctata* EE, constitute encouraging outcomes. This suggests that the botanical effective antifungal concentration (MIC) could be halved when combined.

On the other hand, the most effective inhibitors of mycelial growth of both phytopathogens were *Z. punctata*, *C. gilliesii,* and *L. turbinata* EEs. All three extracts behaved as fungistatic on *P. parasiticum,* while *Z. punctata* and *L. turbinata* EEs killed *V. dahliae* mycelium (fungicidal effect), evidencing that *V. dahliae* vegetative growth was more susceptible to our EEs than *P. parasiticum.*

*In vitro* studies with *Z. punctata* EE at 1.6 mg/mL could not completely inhibit hyphal growth of *Fusarium* species associated with Ear Rot in cereals (13). In contrast, our findings showed that the AA of *Z. punctata* EE, ranging from 1-1.5 mg/mL could completely inhibit mycelial growth of *V. dahliae* and *P. parasiticum*. Results also showed that *Z. punctata* EE MIC values were 2-3 times lower on the spores than on the mycelium of both phytopathogens, consistent with previous findings (13). Considering *C. gilliesii* EE, MIC was similar for conidia germination and mycelial growth of *V. dahliae*. Surprisingly, a complete reduction of *V. dahliae* and *P. parasiticum* mycelial growth was observed with *L. turbinata* EE. However, it did not provide complete control over conidia germination, suggesting a differential effect of extract components on each fungal structure.

Considering all the evaluated EEs, *Z. punctata* EE was the most effective at suppressing spore germination and mycelial growth. Previous research has corroborated the AA of *Z. punctata* EE against other phytopathogenic fungi, attributing this property to polyphenolic compounds, especially chalcone type (4, 13, 23). Considering the difference in the AA observed among the different EEs evaluated, phenols and flavonoid content were quantified, showing that *Z. punctata* EE had the highest content of phenols and flavonoids likely responsible for its potent AA.

Finally, we found that total phenols had the best correlation with mycelial growth inhibition, while flavonoid levels best correlated with inhibition of spore germination. Thus, the AA of studied EEs on conidial germination could be mainly attributed to flavonoid content, while phenols would be responsible for inhibitory effects on mycelial growth.

## Conclusions

This work searched for antifungals of plant origin against pathogenic fungi involved in grapevine trunk diseases and Verticillium wilt of olive. We explored the *in vitro* antifungal properties of five EOs and seven EEs obtained from Argentinian northwest plants. All tested EOs and EEs showed varying AA degrees against both phytopathogenic fungi. This activity depended on plant species, extract type (EO or EE), pathogen identity, and targeted fungal structures. According to our findings, the products obtained from *Z. punctata*, *C gilliesii* and *L. turbinata* were the most effective against *V. dahliae* and *P. parasiticum*, suggesting their potential as biofungicides for integrated disease control. This is particularly encouraging considering absent effective treatments against these two pathogens. Further research should determine antifungal effectiveness of these botanical products in plants and identify their specific antifungal compounds.

**Supplemmentary material**

https://drive.google.com/drive/folders/1O7n5y_WfEk5QOQJzHepnvtvOcieXZhuG?usp=sharing

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M.S is a fellow and N.B is a researcher, both at CONICET, Argentina.