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Research Papers

Agricultural by-products from asparagus and date palm affect *Phytophthora cinnamomi*: potential for control of oak root disease

Rosa LÓPEZ GARCÍA[1], María Teresa HIDALGO FERNÁNDEZ[1], Marta GARCÍA GARCÍA[1], Rocío RODRÍGUEZ-ARCOS[2], Ana JIMÉNEZ-ARAUJO[2], María Socorro SERRANO MORAL[1,*]

[1] *Grupo de Recursos genéticos, mejora y protección del olivo y otros cultivos leñosos (UCOLIVO), Departamento de Agronomía, Universidad de Córdoba, Spain*
[2] *Grupo de Fitoquímicos y Calidad de los Alimentos, Departamento de Fitoquímica de los Alimentos, Instituto de la Grasa (IG-CSIC), Spain*
*Corresponding author. E-mail: a12semom@uco.es

Summary. As society demands healthier food production, agricultural residues could provide sustainable alternatives for plant disease control, as these residues are reservoirs of fungicidal compounds. This study aimed to identify the activity of six plant extracts and their corresponding crude residues against plant pathogen *Phytophthora cinnamomi*. Extracts enriched in bioactive compounds came from residues of asparagus spears (AS), fronds (AF) or roots (AR); olive tree fruit (OF) or leaves (OL), and seeds of date palm fruits (DS). *In vitro* experiments showed that all the asparagus extracts at 0.025 and 0.05% significantly decreased *P. cinnamomi* mycelial growth, with inhibition percentages greater than 75% for AS and AR. The AS, OL and DS extracts at 0.05% inhibited *P. cinnamomi* sporangial production by >84%. Phytotoxicity symptoms were not detected for any of the extracts assessed. *In planta* experiments were carried out to compare effects of soil application of AS and DS organic extracts with their crude residues at 0.05% against disease caused by *P. cinnamomi* on *Quercus ilex* seedlings. The AS residue was statistically more effective than the AS extracts, while DS residue and extract inhibited root rot symptoms to levels similar to the uninfested controls. Therefore, extracts and residues from seeds from date fruits, and residues from asparagus spears, are candidates for control *P. cinnamomi* root rot. Both residues are being tested in an infested field of *Q. ilex* adult trees.

Keywords. Anti-oomycete activity, extracts, phenolic compounds, residues, root rot.

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ORCID:
RLG: 0009-0006-6446-5773
MTHF: 0000-0001-9859-4035
MGG: 0009-0005-2964-9617
RRA: 0000-0002-0003-4345
AJA: 0000-0002-2695-4588
MSSM: 0000-0003-3896-1993

INTRODUCTION

Plants produce many secondary metabolites that have broad biological activity, including antioxidant, anticancer, antibacterial, and antifungal activities, which are related to their chemical structures (Sparg *et al.*, 2004). Previous studies have determined that wild plants are important sources of bioactive compounds, that are rich in saponins and flavonoids and have antifungal

activity against plant pathogens (Cenobio-Galindo *et al*., 2024), including *Fusarium oxysporum* (Tucuch-Pérez *et al*., 2020), and *Phytophthora capsici* (Lujan *et al*., 2021), *P. nicotianae* (Bowers and Locke, 2004), and *P. infestans* (Nagar *et al*., 2017).

Agricultural crops and their by-products are also reservoirs of phytochemicals (Jaramillo-Carmona *et al.,* 2017; Cenobio-Galindo *et al*., 2024; Mrabet *et al*., 2024). Date seeds, which are discarded organic residue, have high contents of polyphenols, and are a rich source of bioactive compounds such as carotenoids, flavonoids, and tocopherols (Mrabet *et al.,* 2024). Similarly, *in vitro* studies have shown that extracts from *Asparagus officinalis* by-products inhibited the mycelial growth of the plant pathogenic fungi *Alternaria tenuissima*, *F. oxysporum*, *Macrophomina phaseolina*, *Rhizoctonia solani* and *P. cinnamomi* (Rosado-Álvarez *et al*., 2014; Desoukey *et al.,* 2020; Romero *et al*., 2024).

Biomass from different plants varies in composition and concentration of secondary metabolites, and in antimicrobial activity (Fuentes-Alventosa *et al*., 2013; Desoukey *et al.,* 2020; Cenobio-Galindo *et al*., 2024). Del Río *et al.* (2003) noted that extracts from *Olea europaea* stems had the highest accumulation of the phytochemicals tyrosol, catechin and oleuropein in cortices, and their use resulted in a more effective inhibition of the mycelial growth of different *Phytophthora* species in comparison with extracts obtained from olive leaves. In contrast, olive leaf extract reduced host plant death caused by *P. cinnamomi* root disease more than extract from olive branches (Romero *et al*., 2024). Therefore, plant byproducts and extracts provide safe and "green" alternatives for management of plant diseases, both in agricultural and natural settings.

Perhaps, there is no better example of a pathogen that affects natural and agricultural systems than *P. cinnamomi*. This soilborne oomycete is one of the 100 worst invasive exotic species (Global Invasive Species Database 2025), and is capable of infecting close to 5000 plants species, including many of importance in agriculture, forestry, and horticulture. This pathogen has serious consequences for environments, biodiversity and substantial economic losses (Burgess *et al*., 2017; Sena *et al.,* 2018). Its impacts are in temperate and subtropical climate regions, including all Mediterranean climate regions (Burgess *et al*., 2017). In South Africa, *P. cinnamomi* severely affects eucalyptus, avocado, macadamia and autochthonous fynbos (Sena *et al*., 2018), while in Europe and California, it is associated with oak decline and chestnut ink disease (Garbelotto *et al.,* 2006; Burgess *et al*., 2017; Sena *et al*., 2018).

Phytophthora cinnamomi is the main causal agent of extensive death of *Quercus* species in the Mediterranean Basin (Brasier, 1996). The pathogen causes severe feeder root necroses with crown wilting, desiccation, and tree defoliation and death (Brasier, 1996). Several methods have been developed to control *P. cinnamomi* oak tree disease, including soil application of calcium amendments (Serrano *et al.,* 2012a), biofumigation with *Brassica* spp. with high content in sinigrin (Ríos *et al.,* 2016), and chemical control using phosphonates (González *et al*., 2020).

Society demands sustainable, healthy and environmental-friendly food production, including a significant reduction in the use of chemical products to control pests. Therefore, the development of new strategies for a sustainable and environmental-friendly biological control of *P. cinnamomi* is urgent. The objective of the present study was to assess biocidal activity against *P. cinnamomi* of six organics extracts that came from olive tree, asparagus or date palm residues. The study aimed to determine: 1) effects of organic extracts on mycelial growth and infectivity of *P. cinnamomi* in *in vitro* experiments; and 2) the comparative effects of organic extracts and their crude residues on development of *P. cinnamomi* root rot *in planta*.

MATERIALS AND METHODS

Plant extracts

Six different organic extracts were used (Table 1). Three were from asparagus crop by-products, from basal portions of spears discarded during processing, and from spears broken or deteriorated during handling (AS), fronds (AF) or roots (AR) of asparagus plants discarded after spear harvesting. Two other extracts were from olive fruit extract obtained during olive oil extractions (OF), or from leaves and twigs resulting from pruning activities (OL). The sixth extract was from discarded seeds from date fruits (DS).

Different extraction protocols were used, depending on the particular plant residues. Extracts from spears (AS), fronds (AF) or roots (AR) residues of the cultivation of *triguero* asparagus from Huetor-Tájar (Granada, Spain), were obtained using the methods of Fuentes-Alventosa *et al.* (2013). Each by-product was first autoclaved at 121 ºC for 2 h in water (1:2 w/v), to obtain an aqueous extract containing most of the soluble bioactive compounds, and the fibrous residue. The aqueous extract was then fractionated by adsorption chromatography and eluted with 40% ethanol aqueous solution as Rosado-Álvarez *et al.* (2014). The resulting extract was concentrated under vacuum and freeze-dried.

The functional organic extract from olive fruits (OF) was obtained using the method of Rodríguez *et al.*

Table 1. The bioactive compounds assessed in this study, from different agricultural by-product extracts.

Agricultural by-product sources		Code	Proportions (%) of phenolic compound	Main bioactive compounds	Reference
Asparagus	Spears	AS	6.33	Rutin (Quercetin-rutinoside)	Fuentes-Alventosa *et al.* (2008)
	Fronds	AF	24.80	Rutin (Quercetin-rutinoside)	López *et al.* (2025)
	Roots	AR	67.83	Protodioscin	Jaramillo-Carmona *et al.* (2017)
Olive	Fruits	OF	6.20	Hydroxytyrosol Tyrosol	Rodríguez *et al.* (2007)
	Leaves	OL	4.46	Oleuropein Dihydroxyphenylglycol	Romero *et al.* (2024)
Date	Seeds	DS	2.56	Pyrogallol	Mrabet *et al.* (2024)

(2007). The aqueous phase liquor generated during olive oil extraction from fruits was purified using an anion exchange chromatography column. The phenolic compounds fraction was eluted with water, and then concentrated using a rotatory vacuum evaporator. The resulting effluent was then purified using polymeric adsorption resin column and eluted with ethanol:water (30:70).

The olive leaf extract (OL) was obtained from dried leaves through aqueous extraction at 90 ºC for 40 min. Due to its high phenolic content (Romero *et al.,* 2024), no additional purification was required. The extract was then concentrated under vacuum and lyophilized (Romero *et al*., 2024).

Date seed extract (DS) was produced from a fine seed powder by extraction with 80:20 (v/v) ethanol:water. After filtration, the resulting liquor was concentrated under vacuum and freeze-dried (Mrabet *et al*., 2024).

Oomycete material

Three *P. cinnamomi* isolates were used. PE2203 was isolated from the rhizosphere of *Quercus ilex* and stored in the fungal collection of the Agronomy Department of the University of Córdoba (Romero *et al.,* 2024). Isolate PA37 was from *Quercus suber* roots, and was stored in the Universidade do Algarve (Portugal). Isolate P7S was from *Q. ilex* roots, and was obtained from the collection of the Agenzia della Regione Sardegna per la Ricerca Scientifica (Italy).

In vitro *experiments*

Effects on mycelial growth. Abilities of organic extracts of agricultural residues to inhibit mycelial growth of *P. cinnamomi* was evaluated. Corn Meal Agar medium (CMA, 17 g L^{-1}) was amended with the different organic extracts at two concentrations (0.025 and 0.05%), selected according to previous tests for AS extract (Romero *et al.,* 2022). All extracts were diluted in water, except for AR extract which was diluted in 70% ethanol before adding it to CMA to achieve a final concentration of 0.02% ethanol. To discard interferences of the solvent and AR extract on *P. cinnamomi* growth, ethanol at 0.02% was also tested (Solvent treatment). Amended CMA was poured into Petri dishes (9 cm diam.; 20 mL per dish). CMA free of extract was used as experimental controls.

Using the methods of Serrano *et al.* (2012a), agar plugs (5 mm diam.) of each *P. cinnamomi* isolate actively growing on 20% Carrot Agar (CA) were placed in the centres of Petri dishes containing the amended or control CMA. Four dishes (replicates) were prepared per isolate, organic extract, and dose, plus the untreated controls. The plates were then incubated at 25 ºC in darkness. Diameters of resulting colonies were measured daily for 5 d, when the colonies of isolates P7S and PA37 covered the entire agar surfaces in the plates, and the trial was concluded. The experiment was repeated three times. Mycelial growth throughout the experiment was expressed as the relative area under progress curve (rAUPC), as calculated using the formula (Campbell and Madden, 1990):

$$rAUPC = \frac{100}{(s_{max} \times t_e)} \times \sum_{i=1}^{n} \frac{(s_i + s_{i+1})}{2} \times \left(t_{i+1} - t_i\right)$$

where: s_i = mycelial growth for observation number *i*,
s_{max}= maximum growth value,
t_i = number of days between *i* evaluations,
t_e = total period of evaluations, and
n = number of evaluations.

Data of rAUPC did not conform to normality, even after applying transformations, so a Kruskal-Wallis One-

Way Nonparametric test was carried out for repetition, isolate, replicate, and the combination treatment-dose as three independent factors. Mean values were then compared using Dunn's test (P <0.05). In addition, percentages of mycelial growth inhibition respect to the untreated experimental controls were calculated. All statistical analysis were carried out using Statistix software 10.0 (Analytical Software).

Effects on sporangial production. The most effective dose (0.05%) of the organic extracts (AS, AF, AR, OH, OF, and DS), based on results from the mycelial growth experiments, was tested for effects on sporangial production, as described by Serrano *et al.* (2012a). Sporangial production was induced as follows: agar plugs (1 cm diam.) of the three *P. cinnamomi* isolates actively growing on Pea Agar (20%) were transferred to empty 6 cm Petri dishes, and were each covered with 7 mL of soil extract (0.1% soil:water), prepared as described in Ribeiro (1978). The dishes were then incubated under constant light at 23 ºC, and the aqueous soil extract was replaced every 24 h. Twenty-four hours before each isolate reached maximum sporangial production, which happened at 56 h for isolate PE2203, and at 72 h for isolates P7S and PA37 (previously determined), the soil extract was replaced by a soil extract amended with the six organic extracts (above, at 0.05%). The extracts were diluted in water, except for asparagus root (AR) extract, which was diluted in 70% ethanol to a concentration of 0.02%. As in the previous experiments, ethanol (0.02%) was also included to detect interference with the AR extract (Solvent treatment). Three plates (replicates) per treatment and isolate were prepared, including three more plates per isolate with unamended soil extract used as negative controls. After the last soil extract changes, all the plates were incubated for 24 h at 23 ºC under constant light, until the negative control for each isolate reached maximum sporangial production. At that time, sporangia were quantified by direct observation using an inverted microscope (Nikon, 400× magnification), and mature sporangia were counted. The experiment was carried out at four different times (repetitions).

Sporangial production values were referenced to the corresponding values of untreated controls, and were expressed as percentage inhibition of sporangial production. These data were analysed using a Kruskal-Wallis non-parametric test, and mean values were compared by Dunn's test (P < 0.05).

Extract phytotoxicity

A previous study demonstrated the lack of substantial phytotoxicity of AS, OH and OL extracts at 0.1 and 0.15% (Romero *et al*., 2024), so plant damage was not expected at dose rates of two or three times less. Therefore, the present study focused on determining the phytotoxic effects of AF, AR and DS organic extracts. Firstly, the extracts effects were assessed on seed germination of *Lupinus luteus*, as described by Romero *et al.* (2024) (Phytotoxicity test 1). Agar medium (1.5%) was amended with each organic extract (AR, AF or DS) at 0.05%, using unamended plates as experimental controls. Plates amended with the 70% ethanol solvent of AR in a final dose of 0.02% were included for a further comparison with AR extract. Seeds of *L. luteus* used for the germination tests were surface sterilized (Serrano *et al.,* 2012b). Six seeds were then transferred to each Petri dish with amended or non-amended (control) agar medium. Five Petri dishes (replicates) were prepared per treatment. All the plates were then incubated at 23 ºC with a 12 h photoperiod. Percentage of seed germination was determined daily for each plate over a 4 d period. Proportions of seed germination for each treatment were analysed by Kruskal-Wallis non-parametric tests, because percentages did not fit normality, and means were compared using Dunn's test (P < 0.05).

A second phytotoxicity test (Phytotoxicity test 2) was carried out to determine effects of the organic extracts on *L. luteus* plant growth. In this case, due to extract availability, the two different experiments were carried out separately, one using asparagus extracts (AF and AR), and the other using DS extract. For these assays, 10-d-old *L. luteus* seedlings produced as described by Serrano *et al.* (2012b) were transferred to seedbed trays containing peat amended with the three organic extracts AR, AF, or DS, each at 0.05%. Trays with peat treated with ethanol (0.02% solvent) and unamended peat were included as experimental controls. Twelve seedlings (replicates) per treatment were planted. All plants were then incubated in a growth chamber at 23 ºC with a 12 h photoperiod, and were watered as required. Phytotoxicity test 2 ended after 37 d, when the plants started to bloom. At that time, plant yields were determined by measuring total plant lengths and fresh weights. Resulting data did not fit normality after applying transformations, so they were analysed by Kruskal-Wallis non-parametric tests, and mean values were compared by Dunn's test (P < 0.05). Each phytotoxicity test was repeated twice.

Effects of extracts and crude residues on host plant root rot

The effects of the two organic extracts that were most effective *in vitro* and in the phytotoxicity experiments, AS and DS, were evaluated on root rot development caused by *P. cinnamomi* using *in planta* experi-

ments. Additionally, effects of both organic extracts enriched in bioactive compounds were compared with the full crude residue used to obtain them: previously dried and shredded discarded asparagus spears (AS-R) and date seeds (DS-R).

A peat: sand substrate (1:1 vol.) was infested with aqueous suspension of isolate PE2203 chlamydospores, as described by Romero *et al.* (2024). Inoculum suspension was adjusted to 250 viable chlamydospores per gram of dry soil. Four days after soil infestation, each treatment (AS or DS extracts and AS-R or DS-R residues) was separately added to the substrate at 0.05% (w/v). Substrate infested and untreated (Control 1), and uninfested and untreated (Control 0) were also prepared as experimental controls. After treatment applications, 1-year-old holm oak seedlings were planted in pots (ten replicates per treatment, each pot containing 2 L of previously prepared substrate, including controls). All pots were incubated in a shade-house. One week after the plants were potted, all pots, including the controls, were placed in plastic trays without drainage (five pots per tray), and the trays were partially filled with tap water maintaining flooding for 2 d each week until the end of the experiment (Serrano *et al.*, 2012a). The experiment was repeated twice, starting in October 2023 for repetition 1 and January 2024 for repetition 2. For both repetitions, severity of foliage symptoms was assessed each week for each plant, using a 0 to 4 scale, according to percentages of yellowing, wilting, desiccation, and defoliation: where 0 = 0%, 1 = 1–33%, 2 = 34–66%, 3 = more than 67%, and 4 = dead tissue; Romero *et al.,* 2007). The experiments ended when the mean foliar symptom severity values from Control 1 (infested and untreated) became greater than 2.5, which happened at 14 weeks after treatment for experiment repetition 1, and 27 weeks after treatment for repetition 2. At those times, plants were removed from substrate, and severity of root symptoms was evaluated using a similar 0 to 4 scale according to percentage of root necrosis (Romero *et al.*, 2007).

Evolution of foliar symptom severity during the two experiments was expressed as relative areas under disease progression curves (rAUDPC), calculated using the formula outlined above. Data of rAUDPC and severity of root symptoms did not conform normality after applying transformation, so a Kruskal-Wallis One-Way Non-parametric test was carried out considering the repetitions and treatments as independent factors. Mean values were compared using Dunn's test ($P < 0.05$). In addition, root segments from all plants, including from Control 1 and Control 0, were washed and plated onto PA-NARH medium for re-isolation of the pathogen (Romero *et al.*, 2024).

RESULTS

In vitro *experiments*

Effects on mycelial growth: Statistical analyses showed differences of rAUPCs among the three isolates ($F = 8.77$, $P = 0.0002$), so the isolate data were analysed separately. For each isolate, significant differences were observed among treatments ($F = 73.65$, $P < 0.0001$ for PE2203; $F = 62.57$, $P < 0.0001$ for PA37; $F = 56.9$, $P < 0.0001$ for P7S;), while no differences were detected among repetitions or replicates. Although no effects were recorded for olive and date palm extracts, the three organic extracts of asparagus (AF, AS and AR) at both doses tested (0.025 and 0.05%) induced less mycelial growth than the untreated control and the solvent, except for AF at 0.025% for the Italian isolate (P7S) that did not differ from the controls (Figure 1).

Since the statistical analysis of the percentage of mycelial growth inhibition did not show significant differences among repetitions ($F = 0.83$, $P = 0.4373$) and among the isolates ($F = 1.9$, $P = 0.1511$), Figure 2 shows average values for the three isolates together. The most inhibitory extracts were AS and AR at both assessed doses, reaching inhibition percentages greater than 77%.

Effects on sporangial production: Figure 3 shows mean values of the percentages of inhibition of sporangial production after treatment with each organic extract at 0.05%. Differences were found among treatments ($F = 23.1$, $P < 0.0001$), but not among isolates ($F = 1.44$, $P = 0.2395$), repetitions ($F = 1.85$, $P = 0.1385$) or replicates ($F = 0.46$, $P = 0.6338$). The aqueous extracts AS, OL and DS, inhibited *P. cinnamomi* sporangial production by greater than 84%, while AF and OF extracts gave lesser mean inhibition percentages (respectively, 27.4 and 43.1%) (Figure 3). For the ethanol extract AR, although it induced the greatest sporangial inhibition (93%), this was not different from its solvent treatment.

Extract phytotoxicity

Figure 4 shows evolution of the percentage of *L. luteus* seed germination under the influence of different organic extracts AF, AR or DS at 0.05% in Test 1. Germination percentages did not differ ($P > 0.05$) from the untreated controls at the end of the experiment. Phytotoxicity symptoms, including organ deformation, discolouration and/or necroses of plant tissues, were not observed from any treatment in Test 2. However, differences were detected among the treatments for mean plant total length ($F = 6.13$, $P = 0.0002$) and mean plant fresh weight ($F = 7.06$, $P < 0.0001$). AR and AF induced

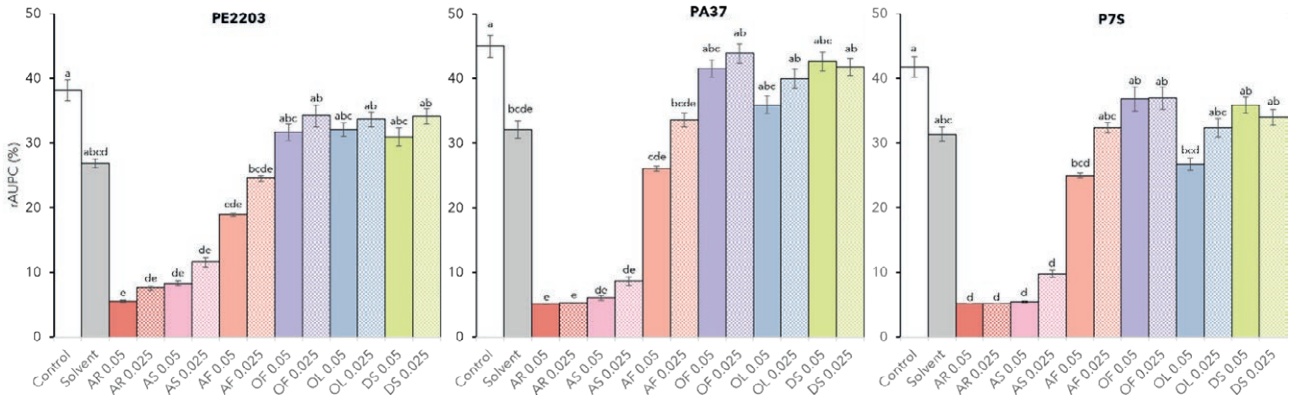

Figure 1. Means and standard errors of relative areas under progression curves (rAUPC) for mycelial growth (after 4 d) of three *Phytophthora cinnamomi* isolates (PE2203, PA37 and P7S) in medium amended with organic extracts (AR, AS, AF, OF, OL and DS) at 0.025 and 0.05%, solvent (0.02% ethanol), and in unamended media (control). Bars accompanied by different letters indicate differences according to Dunn´s tests at $P < 0.05$. AR: Asparagus Root extract, AS: Asparagus Spear extract, AF: Asparagus Frond extract, OF: Olive Fruit extracts, OL: Olive Leaf extract, DS: Date Seed extract.

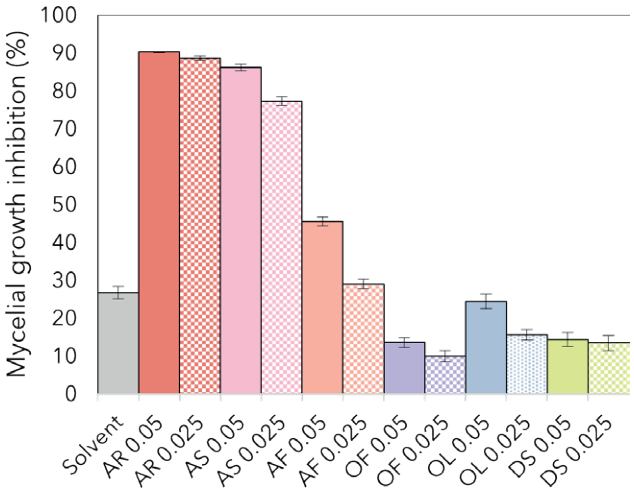

Figure 2. Mean and standard error of percentages of mycelial growth inhibition of three *Phytophthora cinnamomi* isolates (PE2203, PA37 and P7S) in media amended with the six organic extracts (AR and its Solvent, AS, AF, AL, OF and DS) at doses of 0.025 or 0.05%. AR: Asparagus Root extract, AS: Asparagus Spear extract, AF: Asparagus Frond extract, OF: Olive Fruit extract, OL: Olive Leaf extract, DS: Date Seed extract.

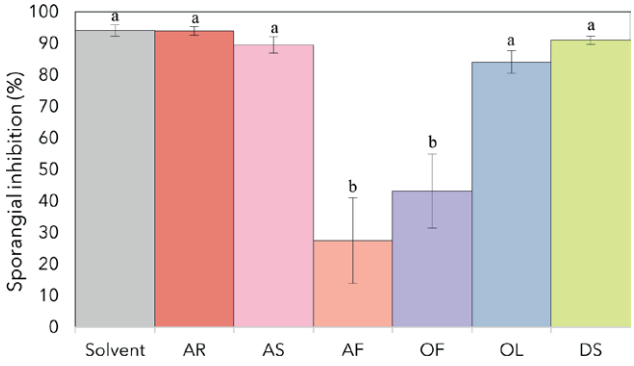

Figure 3. Mean (± standard error) of *Phytophthora cinnamomi* sporangial production inhibition induced by six organics extracts (AR, AS, AF, OF, OL and DS) at 0.05% doses, and solvent (ethanol 0.02%) for AR extract. Bars accompanied by different letters differ, according to Dunn's test ($P < 0.05$). AR: Asparagus Root extract, AS: Asparagus Spear extract, AF: Asparagus Frond extract, OF: Olive Fruits extract, OL: Olive Leaf extract, DS: Date Seed extract.

reductions in plant yields in comparison to the untreated controls (Figure 5).

Root rot development

Plants growing in infested and untreated substrate (Control 1) developed foliar symptoms (leaf yellowing and wilting) typical of *P. cinnamomi* oak tree infections in field and pot experiments (Sánchez *et al*., 2002; Romero *et al*., 2007). Statistical analyses of data of development of foliar symptoms (rAUDPC) indicated significant differences among treatments ($F = 4.96$, $P = 0.0004$), but not between repetitions ($F = 1.13$, $P = 0.2908$), so the rAUDPCs for both repetitions were combined in a single analysis. As shown in Figure 6, plants treated with residues and extracts from asparagus or date palm had reduced rAUDPCs compared to inoculated and untreated plants (Control 1), not differing among them nor with the negative controls (uninfested, untreated).

Differences in root symptoms were detected among treatments ($F = 14.1$, $P > 0.0001$), but not for repetitions ($F = 0.86$, $P = 0.3547$), so data of both repetitions were combined in a single analysis (Figure 7). Plants grow-

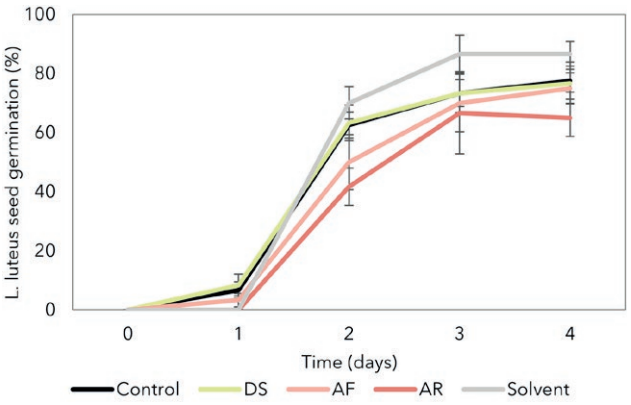

Figure 4. Mean (and standard error) of *Lupinus luteus* seed germination percentages in media amended with Asparagus Root organic extract (AR), Asparagus Frond organic extract (AF) or Date Seed organic extract (DS) at 0.05%, and in unamended media (controls).

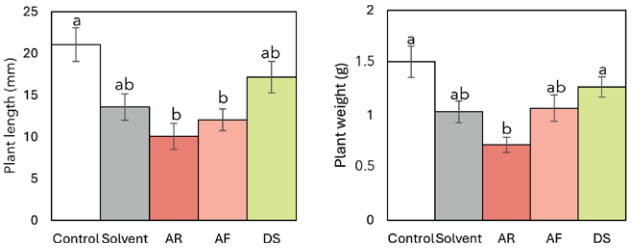

Figure 5. Mean plant lengths (and standard errors) (left), and mean plant fresh weights (right) for *Lupinus luteus* seedlings growing in peat amended with Asparagus Root organic extract (AR), Asparagus Frond extract (AF) or Date Seed extract (DS) at 0.05% concentrations, and solvent (0.02% ethanol) and unamended control. Means accompanied by different letters are different, according to Dunn's test ($P < 0.05$).

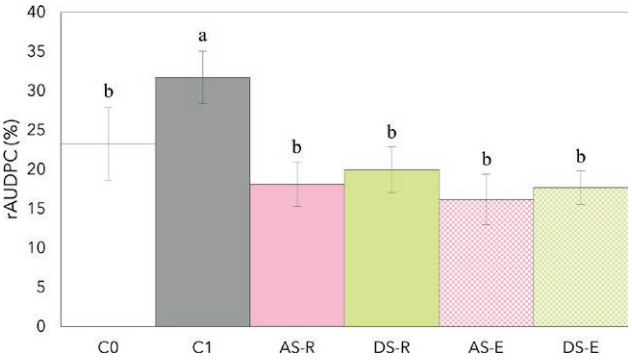

Figure 6. Mean (and standard error) areas under disease progress curves (rAUDPC) of the severity of foliar symptoms on holm oak seedlings growing in substrate infested and treated with the asparagus or date palm residues and organic extracts, or with infested and untreated substrate (C1) or uninfested and untreated substrate (C0). Means accompanied by different letters are different according to Dunn's test ($P < 0.05$).

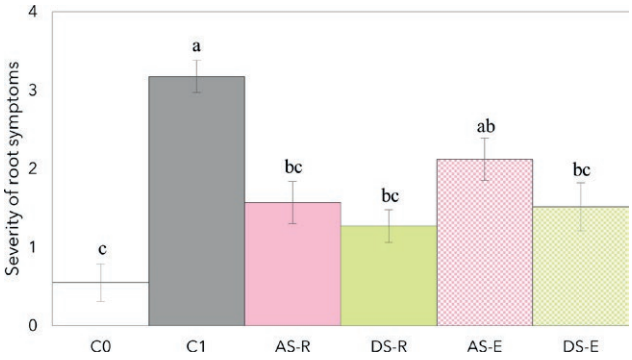

Figure 7. Mean (and standard error) root symptoms severity scores for holm oak seedlings growing in substrate infested and treated with asparagus or date palm residues (AS-R and DS-R) or organic extracts (AS-E from asparagus or DS-E from date palm), and from infested and untreated substrate (C1) and uninfested and untreated substrate (C0). Means accompanied by different letters are different according to Dunn's test ($P < 0.05$).

ing in substrate treated with date seed extract or residue (DS-E or DS-R), or with asparagus spears residue (AS-R), developed significantly less severe root symptoms than the infested and untreated plants (Control 1), and were not different from untreated and uninoculated holm oaks (Control 0). AS-E plants did not differ from Control 1, and had significantly more severe root symptoms than plants in Control 0 (Figure 7). *Phytophthora cinnamomi* was re-isolated from all plants growing in infested substrate that was amended or not amended with extracts and residues (isolation proportions 36.1 to 72.2%), but never from plants growing in uninoculated substrate (Control 0).

DISCUSSION

This study assessed anti-oomycete activity of extracts obtained from by-products of *triguero* asparagus from Huétor-Tájar (Granada), olive or date palm residues in experiments across the main stages of the *P. cinnamomi* pathogenicity cycle (mycelial growth, sporangial production, and disease development). Comparisons were also made between the most effective organic extracts enriched with bioactive compounds and the residues for effects on root disease development. Although previous studies have shown that extracts from different plant species had antimicrobial activity against phytopathogenic fungi (Rosado-Álvarez *et al.*, 2014; Tucuch-Pérez *et al.*, 2020) and oomycetes (Nagar *et al.*, 2017; Lujan *et al.*, 2021; Cruz *et al.*, 2024), the studies have focused on *in vitro* experiments (Nagar *et al.*, 2017; Cruz *et al.*, 2024). The present study included *in vivo* tests to assess

the antimicrobial activity of organic plant extracts and their crude residues in a realistic setting. Additionally, the study has provided evidence of effectiveness of date palm and asparagus agricultural by-products for inhibiting *P. cinnamomi* infectivity and *in planta* disease development, indicating their potential for management of diseases caused by this pathogen.

Results from the *in vitro* experiments showed that the organic extracts most inhibitory to *P. cinnamomi* mycelial growth and sporangial production were those from asparagus spears (AS), olive leaves (OL) or date palm seed (DS). There was also a clear differential effect on pathogen inhibition depending on the origin of the residues within asparagus plants and olive trees. Extracts from asparagus spears and roots, at doses of 0.025 and 0.05%, strongly inhibited *P. cinnamomi* mycelial growth, while extract from asparagus fronds were not effective. For sporangial production, a similar response occurred for plant biomass. Inhibition percentages greater than 84% resulted from extracts from asparagus spears (AS) or olive leaves (OL) at 0.05%, while AF, AR and OF were not inhibitory. Sporangial production is directly related to zoospore release, so inhibition of these reproductive structures reduces the infectivity of *P. cinnamomi*. Other research has also reported fluctuating fungicidal activity linked to plant biomass from compounds used against the fungal pathogens *Botrytis cinerea, A. tenuissima, F. oxysporum, M. phaseolina, R. solani*, as well as *P. cinnamomi* and *P. agathicida* (Lawrence *et al*., 2019; Desoukey *et al*., 2020).

Differential behaviour associated with plant biomass could be explained by the extract phenolic profiles, since the composition and concentration of bioactive compounds in plants is related to the particular plant organ (Lawrence *et al*., 2019; Desoukey *et al*., 2020), phenological phase (Ríos *et al*., 2016), geographic location, cultivar, and/or nutritional state (Borjan *et al*., 2020), as well as extraction method (Sales *et al*., 2016). For instance, AS inhibited *P. cinnamomi*, but AF was ineffective, although flavonoid rutin (quercetin 3-O-rutinoside) was the major phytochemical, and the asparagus frond extract had a total phenolic compound concentrations that was greater than the extract from asparagus spears extract (López *et al.,* 2025). These results demonstrate the importance of correct selection plant biomass to effectively control plant pathogens. Dose should be correctly chosen, since the lower activity for reducing sporangial production of *P. cinnamomi* shown in the present study for olive fruit extract in comparison to olive leaf extract, could disappear with increasing extract dose. Romero *et al.* (2024) reported that OF and OL gave sporangium inhibition of greater than 83% at doses of 2 or 3 times greater (0.1 and 0.15%) than those used in the present study.

For asparagus root extract, although it did not affect *L. luteus* seed germination or plant yield in the phytotoxicity tests, effects on *P. cinnamomi* sporangial production from the asparagus root extract could not be differentiated from those of the solvent. Further research is required to confirm if other solvents could be used to discriminate asparagus root extract effects against sporangial production of *P. cinnamomi* without phytotoxic effects.

Limited data are available on the fungicidal activity of date palm extracts, with most of studies being focused on food-based and human pathogens (Al-Tamimi *et al*., 2021). Therefore, the present study results provide new evidence showing that ethanolic extract from date palm seed had high *in vitro* activity against *P. cinnamomi*, reducing sporangial production (91% inhibition). Conversely, no effect on mycelial growth was observed. Bokhari and Perven (2012) reported that different leaf and pit extracts from *Phoenix dactylifera* had varying degrees of mycelial growth inhibition for pathogenic *Fusarium, Aspergillus, Trichoderma* and *Alternaria* but no oomycetes were assessed in their study. In general, *in vitro* data have limited value for predicting field efficacy, while *in planta* results can provide more reliable data.

The *in planta* experiments showed that date fruit seeds effectively reduced root rot development in *Q. ilex* seedlings, both as extract and as crude residue. Conversely, while the asparagus crude residue reduced severity of root symptoms, the extract from asparagus spears was effective only *in vitro* but not *in planta*. Romero *et al.* (2024) observed reductions in *P. cinnamomi* sporangial production and chlamydospore viability when treated with extract from asparagus spears (at 0.15%), resulting in low mortality of lupin plants. The different results obtained in the present study, when testing the efficacy of asparagus spear extract *in planta*, may have been due to the low dose tested. Additionally, the differences found in the present study between *in vitro* and *in planta* experiments for asparagus spears extracts could have been due to soil factors that can modify effectiveness of compounds and host plant responses (Goufo *et al*., 2010; Serrano-Pérez *et al.,* 2021), compared with the *in vitro* experimental conditions where there are direct pathogen-extract interactions. Outcomes from this study have demonstrated the importance of confirming *in vitro* results by means of *in planta* experiments. On the other hand, the differential *in planta* behaviour found between asparagus spear extract and its crude residue could be explained by different causes. Firstly, the compositions of extract and residue are probably different. Although extract has greater total concentration of phenolic compounds, crude residue has other chemical components and fibrous biomass, that may influence on effectiveness (Desoukey *et al*.,

2020; Ahmad and Matsubara, 2020). Secondly, rapid degradation of bioactive compounds from asparagus spear extract could reduce period for inhibiting *P. cinnamomi* infectivity, compared to slow release from crude residues. *Brassica carinata* effectiveness against *P. nicotianae* was also dependent on volatile compound release and exposure time (Serrano-Pérez *et al.,* 2017; 2021).

The date seed extract assessed in the present study directly affected *P. cinnamomi* by reducing sporangial production, and (presumably) the quantity of zoospores within sporangia. This effect translated into decreased root rot symptoms on *Q. ilex* seedlings infected by the pathogen. Previous studies have also demonstrated the effectiveness of agricultural by-products extracts, including those from carob and pomegranate peel, for reducing incidence of olive anthracnose by inhibiting conidium germination in *Colletotrichum* spp. (Pangallo *et al.,* 2017; Antón-Domínguez *et al*., 2025). However, several studies have reported dual action of plant extracts against phytopathogens, with direct antimicrobial activity combined with indirect action as resistance inducers (Hassan *et al*., 2009; Saltos-Rezabala *et al*., 2022; Antón-Domínguez *et al.,* 2025). Saltos-Rezabala *et al.* (2022) observed that although thyme essential oil had direct antifungal effects against *Alternaria linariae*, it did not eradicate the pathogen, but gave effective control of the pathogen by increasing defences of tomato plants. Recent studies (Serrano, personal communication) have demonstrated preventive effects of pyrogallol against *P. cinnamomi* root rot of *Q. ilex* seedlings. Pyrogallol is the main phenolic compound in date seed extract and residue. A double action mode could be involved from treatments with date seed extracts. These results highlight the need for further research to determine possible activity of date seed residue and extract as resistance inducers during pathogen infection progression.

The present study results highlight the effectiveness of full crude residues from asparagus spears and date seeds, for control of root rot caused by *P. cinnamomi*. In addition, these treatments have advantages in comparison to extract applications, such as organic matter contribution to soil structure, the possible synergic effects with other antagonistic microorganisms (Serrano-Pérez *et al*., 2017; 2021), and the simple and low-cost treatment application, that make crude plant residues effective alternatives for control of soilborne pathogens.

CONCLUSIONS

This study provides a first approach to development of a potentially environmentally friendly approach to control *P. cinnamomi* root rot on *Quercus* species, based on soil applications of extracts and residues from agricultural crops. In particular, the promising results obtained for reducing *P. cinnamomi* infectivity and root rot development resulting from treatments of asparagus spear residue and extracts from seeds of date palm fruits, indicates that they are candidates for plant pathogen control. Their use would also promote recycling of residues and "circular economies". Effects of asparagus spears and date seed residues against *P. cinnamomi* are currently being evaluated under natural conditions in a pathogen infested field of mature *Q. ilex* trees.

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