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Research Article

Ecological control of mycotic pathogens in tomato crops - alternatives to synthetic pesticides

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Abstract

The pathogens *Alternaria solani* and *Fusarium oxysporum* f. sp. *lycopersici* are of significant interest from a pathogenic perspective in the context of tomato cultivation. This study focuses on evaluating the fungicidal and fungistatic effects of different synthetic substances and natural compounds on the development of these two investigated pathogens. The fungicidal agents employed comprised fosetyl aluminum at a concentration of 0.3%, azoxystrobin at 0.2%, and metiram at 0.3%, while the natural extracts investigated included those derived from European birthwort, celandine and sage, each tested at concentrations of 0.5%, 2%, 9%, and 15%. The assessment of mycelial growth inhibition was conducted utilizing Vincent's formula. Additionally, the total polyphenol content (TPC) within the extracts was determined via the Folin-Ciocalteu spectrophotometric method in accordance with Frum *et al.* (2022). Furthermore, the antioxidant capacity of the extracts was evaluated using the DPPH radical scavenging method (2,2-diphenyl-1-picrylhydrazyl) and high-performance liquid chromatography (HPLC). Our research findings yielded noteworthy results, specifically, extracts derived from sage and celandine, particularly when present at a concentration of 15%, exhibited a fungistatic effect. This effect was particularly remarkable when compared to the performance of the synthetic fungicide azoxystrobin at a concentration of 0.2% when challenged with the *Alternaria* pathogen. These results suggest the potential utility of sage and celandine extracts as eco-friendly alternatives for mitigating fungal pathogens in tomato crops, warranting further investigation and consideration within agricultural practices.

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Keywords: plant extracts; pesticides; tomato crops

Introduction

The concept of ecological agriculture is a key component of sustainable agriculture, where the use of natural biocidal substances is recommended alongside other non-polluting conventional cultural practices (Shi, 2002).

The use of extracts obtained from medicinal plants for the control of horticultural crops' postharvest pathogens is an essential field of research (Jafarzadeh *et al*., 2020). The higher plants and shrubs family, especially tropical flora is a potential source of naturally produced inhibitory chemicals (Malik *et al*., 2016). The successful application of natural products derived from medicinal plant extracts, such as volatile chemicals, essential oils, and phenolic compounds, has been effectively implemented in the control of postharvest diseases of stored fruits and vegetables (Tripathi *et al*., 2007; Azwanida, 2015).

Pesticides have been the most effective method of controlling mycotic pathogens in tomato crops in greenhouses and protected spaces (Amoako Ofori *et al*., 2022). Repeated use of these substances can lead to resistance and residues in soil and water (Gevao *et al*., 2000). This is why it is imperative to develop integrated protection programs in which the interspersed application of synthetic pesticides with those of a biological nature extracted from different medicinal plants is recommended (Marrone, 2019).

The use of biocontrol options such as plant extracts obtained by advanced extraction methods (ultrasound-assisted extraction - UAE; microwave-assisted extraction - MAE and supercritical fluid extraction - SFE) has long been argued to be more sustainable and appropriate, support this and show the use of plant extract-based biopesticides can effectively control pests and can be integrated into sustainable agricultural practices (Tembo *et al*., 2018). They also demonstrated that plant bioformulations used in vegetable crops could support similar yields to those where synthetic pesticides have been used. Also, Kumar and Chandel (2018), tested the efficacy of eight extracts from different plant species against *Podosphaera pannosa* (powdery mildew of rose) under *in vitro* conditions. The most effective treatment was *Allium sativa* L. extract with a conidial germination inhibition area of 66.65%, followed by *Sapindus mukorossi* L. extract.

Another *in vitro* experimental study aimed at testing the antifungal capacity of five plant extracts against the following pathogens: *Penicillium expansum*, *Botrytis cinerea*, *B. allii*, *Monilinia laxa*, *M. fructigena*, *Plasmopara viticola*. Of the five biocontrol options, ethanolic extracts of *Salix alba* and *Artemisia absinthium* were found to be the most effective, resulting in total inhibition of spore germination at a concentration of 1000 mg/L. Moreover, the two plant extracts showed relatively low toxicity in the ecotoxicological test on *Daphnia pulex* (Andreu *et al*., 2018).

Botanical pesticides have become extremely popular with the increasing demand for organic products. There are also studies that show the pesticidal capacity of plant extracts from species such as *Nicotiana tabacum*, *Ryania speciosa*, *Tanacetum cinerariifolium*, *Azadirachta indica* or *Schoenocaulon officinale*. The mechanism of action of secondary metabolites from these plant species is based on both their fungicidal/bactericidal effect and their fungistatic/bacteriostatic effect. These formulations are therefore ideal candidates for synthetic pesticides that can have extremely harmful effects on the environment (Lengai *et al*., 2020).

Aromatic plants and herbs have been extensively investigated as potential sources of natural compounds with medicinal and antimicrobial activity. The genus *Salvia* (sage) includes nearly 900 species worldwide. These species are known for their pharmacological properties and have been used as food, flavoring agents, perfumes, and drugs since ancient times. The compositions of essential oils and extracts of *Salvia fruticosa*, *S. canariensis*, *S. argentea*, *S. spinosa* and *S. officinalis* have been reported (Longaray Delamare *et al*., 2007). *S. officinalis* on phytopathogens such as *Botrytis*, *Penicillium*, *Aspergillus*, *Cladosporium*, *Fusarium* and *Phytophthora* spp. have been demonstrated *in vitro* (Widmer and Laurent, 2006; Wilson *et al*., 1997).

Aristolochia is a large genus belonging to the Aristolochiaceae family. The genus *Aristolochia* consists of between 450 and 600 species that grow in temperate and tropical climates around the world. They are mostly cultivated as ornamentals, but most species are also popular medicinal species. Several studies have analyzed the components of different species of *Aristolochia*. The stem of *Aristolochia trilobata* L. contains a carboxylic acid ester (6-methyl-5-hepten-2-yl acetate), terpenes (limonene, linalool and *p*-cymene), sesquiterpene (bicyclogermacrene) and sesquiterpenoid (spathulenol) (Santos *et al.*, 2014). Various iso-quinoline alkaloids have been isolated from the aerial parts of *Aristolochia constricta* Grisebach (Rastrelli *et al.*, 1997; Shi *et al.*, 2004). *C. majus* L. is a species of papaveraceae and chelidonine (CHE) is one of its most important alkaloids. CHE is a natural benzophenanthridine alkaloid, and the pharmacological effects of alkaloids are widely recognized, including selective inhibition of PKC, and anti-inflammatory and antitumor qualities (Sowa *et al.*, 2018; Borghini *et al.*, 2015; Gilca *et al.*, 2010). Plant extracts and these purified compounds show antibacterial, antiviral, or antifungal action both *in vitro* and *in vivo* (Ulrichova *et al.*, 2001). Their properties were mainly attributed to alkaloids, flavonoids, and phenolic acids (Nawrot *et al.*, 2007). The main alkaloids in the extracts are chelidonine, chelerythrine, sanguinarine, coptisine, and berberine (Sárközi *et al.*, 2006).

In the present study, we aimed to determine the antifungal effect of some extracts of sage (*Salvia officinalis*), European birthwort (*A. clematitis*), and greater celandine (*C. majus*) against the pathogens *Fusarium oxysporum* f. sp. *lycopersici* and *Alternaria solani*.

Materials and Methods

Microwave-assisted extraction was used in the process of obtaining plant extracts from *A. clematitis*, *S. officinalis,* and *C. majus* species. The solvent used was a binary mixture of distilled water and ethyl alcohol, in a ratio of 50:50 v/v. Before being subjected to extraction, the plant material was drying for 5 days at a temperature of 40 °C and grinding for 3 min at 6000 RPM.

Microwave Assisted Extraction (MAE)

For this, a microwave extractor model NEOS-GR (Microwave Extraction System from Milestone Inc) was used, the extract being obtained at the following experimental parameters: total power delivered to the medium was 220 W, for 10 minutes, temperature 80 °C.

Determination of total polyphenol content (TPC)

Into a test tube add 0.4 mL sample solution, 1 mL of Folin- Ciocalteu reagent, 15 mL of purified water and 2 mL of 290 g/L sodium carbonate solution, shake for 10 minutes and keep at 40 °C for 20 minutes in the water bath. Cool and measure the absorbance at 760 mn.

Calibration curve: Into a 25 mL volumetric flask, add 1, 2, 3, 4 and 5 mL of a 1 mg/mL gallic acid standard solution and bring to mark with metanol. The obtained solutions are treated the same as the samples.

The results will be expressed as mg Gallic Acid Equivalents (GAE)/mL extract (Frum *et al*., 2022).

Determination of antioxidant capacity (DPPH) free radical scavenging assay (RSA)

A stock solution of 25 µg/mL DPPH in methanol was prepared and kept at a low temperature and in the dark for 2 h before usage. 970 µL of DPPH stock solution were added onto 30 µL of sample solution. The absorbance was recorded at 517 nm, using a Shimadzu UV 1900 spectrophotometer and the results were expressed as (%). The calibration curve was linear for the range of DPPH concentrations of 0.50 - 250 µg/mL (Georgescu *et al.*, 2022).

The DPPH radical scavenging activity was determined by using the following formula:

$$RSA\ (\%) = \frac{C_0 - C_1}{C_0} \cdot 100$$

Where:

RSA = DPPH radical scavenging activity (%),

C_0 = concentration of the DPPH stock solution (µg/mL),

C_1 = DPPH concentration in the sample (µg/mL).

Phenolic compounds (HPLC)

The HPLC-UV analysis for the identification and quantification of several phenolic compounds was carried out by using a Shimadzu SCL-40 HPLC system equipped with a degasser, quaternary pump, photodiode array detector, thermostatted column oven and autosampler. The column used was Nucleosil C18 (250 mm x 4.6 mm, i.d. 5 µm). The oven temperature was 25 °C. The elution was performed by using three mobile phases: A, purified water; B, methanol; and C, 96:4 (V/V) purified water: acetic acid in a gradient program: 15% B and 85% C at 0 min, 75% A and 25% B at 15 min, 15% A and 85% B at 20 min, 40% A and 60% B at 40 min followed by column conditioning. The flow rate was 0.5 mL/min for the first 15 min and 0.8 mL/min from minute 15. The detection was performed at 280 nm for gallic acid, (+)-catechin, syringic acid, and cinnamic acid, 306 nm for resveratrol, 330 nm for caffeic acid, chlorogenic acid, and ferulic acid, and 360 nm for rutin and quercetin and the injection volume was 5µL. The standards of gallic acid (purity > 99%), (+)-catechin (purity >98%), ferulic acid (purity > 99%), syringic acid (purity > 95%), cinnamic acid (purity > 99%), caffeic acid (purity > 99%), chlorogenic acid (purity >99%) resveratrol (purity > 99%), quercetin (purity > 95%), and rutin (purity > 94%) were purchased from Sigma-Aldrich (Frum *et al.*, 2022).

Antifungal activity

Pure cultures were obtained through the hyphal tip method and single spore subculture techniques (Mardare *et al.*, 2015). Isolation and obtaining pure colonies of the *F. oxysporum* f. sp. *lycopersici* and *A. solani* pathogenic agents were performed *in vitro*, on a nutrient medium with potato dextrose agar (PDA, potatoes 20 g, glucose 20 g, agar 20 g), then incubated at 25 °C for 14 days.

In vitro evaluation of fungicides and extracts

The efficacy of fungicides was tested against *A. solani* and *F. oxysporum* f. sp. *lycopersic*i for radial growth inhibition on the Potato dextrose agar medium using the poisoned food technique under in vitro conditions. Twenty ml of poisoned medium was poured into each sterilized Petri plate. A suitable check was maintained without the addition of fungicides. A mycelial disc of 5 mm taken from the periphery of 14 day old colony was placed in the center of Petri and incubated at 24±1 °C for 14 days and three replications were maintained for each treatment. The diameter of the colony was measured in two directions and the average was recorded. In order to establish a notable inhibitory percentage, four different concentrations were used, namely: 0.5%, 2%, 9% and 15%, the observations being recorded at 3, 5, 7, 10 and 14 days respectively.

Also, for the validation of the results and reporting to the conventional protection methods, *in vitro* tests were carried out using synthetic pesticides in different concentrations, namely fosetyl aluminum 0.3%, metiram 0.3% and azoxystrobin 0.2%. The diameter of the colony was measured in two directions and an average value was recorded. Percent inhibition of mycelial growth of the fungus was calculated by using the formula by Vincent (Vincent, 1947).

$$I = \frac{C - T}{C} x100$$

Where:

I = Percent inhibition,

C = Radial growth in control,

T = Radial growth in treatment (fungicide).

Data analysis

Data were processed using one-way ANOVA, followed by Šídák's multiple comparisons test. Processed data were expressed as mean ± standard error (SE). This analysis was performed using GraphPad Prism 9.0.0.0 software.

Results

Characterization of the used extracts

Analysis of polyphenols (TPC)

The total content of polyphenols in the extracts used varied depending on the plant species chosen and the extraction method used (Table 1). In the case of the extract obtained by the micro-assisted technique from sage, the highest amount of polyphenols was recorded, namely, 8.46 mg GAE/mL extract, followed by the extract obtained from *A. clematitis* with a value of 5.12 mg GAE/mL extract. Table 1 shows that the lowest amount of polyphenols was recorded in the extract obtained from *C. majus* with a total polyphenol content of 3.97 mg GAE/mL extract.

Table 1. Variation of total polyphenol content and antioxidant potential of the extracts

Plant extract	TPC (mg/ml GAE)	DPPH %
A. clematitis	5.12	77.81
C. majus	3.97	79.27
S. officinalis	8.46	85.43

Antioxidant activity testing by the DPPH and HPLC methods

According to the results shown in Table 1 and Table 2, the best antioxidant capacity and chemical composition were recorded in the extract obtained from *S. officinalis* L., followed by the extract from *C. majus* L. and *A. clematitis* L.

Table 2. The amount of phenolic compounds (µg/mL extract) obtained from *A. clematitis* L., *S. officinalis* L. and *C. majus* L

Phenolic compound	*A. clematitis*	*S. officinalis*	*C. majus*
	µg/mL extract		
Gallic acid	0.36	3.66	1.87
Catechin	8.21	446.04	13.87
Syringic acid	0.56	1.31	0.13
Cinnamic acid	20.98	0.63	1.70
Resveratrol	0.47	0.13	0.11
Caffeic acid	2.57	1.41	2.82
Ferulic acid	0.32	3.28	0.41
Chlorogenic acid	0.27	2.18	0.21
Rutin	4.53	18.41	0.18
Quercetin	40.09	16.57	0.46

In vitro evaluation of the fungicidal potential of plant extracts

Laboratory tests of biotic activity allowed us to determine the direct effect of plant extracts on the growth dynamics of the fungi. The pathogen *A. solani* that was grown on PDA (potato-glucose agar) culture medium forms whitish colonies with radial development from the point of inoculation. In the central area, the mycelial hyphae show a dark brown colour on a radius of 1.5-1.7 cm. Mycelial hyphae are brown, septate, richly branched, and often intertwined. On this culture medium, the growth of the fungus is very fast; in 4 days, it reaches a diameter of 4.0/3.6 cm, and after 10 days the surface of the Petri dish is completely covered (Figure 1a). In our *in vitro* observations. We also noticed a change in the colour of the colonies from dark brown with white concentric groupings to uniform olive in the whole mass after about two weeks. Being a saprophytic, the fungus does not form pycnidia brown, segmented, fasciculated conidiophores being directly differentiated from the mycelial hyphae. On these conidiophores, brown, muriform conidia are formed (provided with transverse septa and with 1-2 incomplete longitudinal or oblique septa), short pedunculate (Figure 1b).

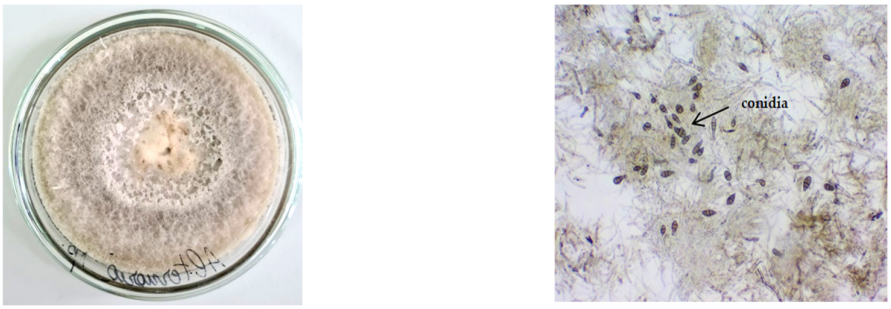
Regarding the antifungal action of the extracts of celandine, sage, and European birthwort, four different concentrations were used, namely: 0.5%, 2%, 9%, and 15% respectively. The observations regarding the growth of colonies were recorded at 3, 5, 7, 10 and 14 days respectively. The obtained results were correlated with the total content of polyphenols components, and with the data from the specialized literature. From the figure 3 (G1-G4) directly proportional relationship is observed between the percentage of inhibition and the concentration of sage extract. The European birthwort extract showed an obvious fungistatic effect at the 15% concentration, the result being the same in the case of the 15% celandine extract Figure 3 (C1-C4 and E1-E4).

The pathogen *F. oxysporium* f.sp. *lycopersici* grown on PDA culture medium (potato-glucose agar) forms whitish colonies with a radial development from the point of inoculation. Unlike the pathogen *A. solani*, this fungus does not show colour differences in the morphology of the colonies. The mycelial hyphae are white-pinkish, septate, richly branched, and often intertwined. On this culture medium, the growth of the fungus is very fast, in 4 days it reaches a diameter of 4/4 cm, and after 10 days the surface of the Petri dish is completely covered (Figure 2a). Being a hyphomycete, the fungus does not form conidiomes on mycelial hyphae, directly differentiating into hyaline, septate conidiophores. Two types of conidia were formed on the conidiophores: ovoid or fusiform microconidia, unicellular, chained, or agglomerated; macroconidia cylindrical in the central part, narrowed and curved at the extremities, 4-5-septate, forming yellowish-pink agglomerations (Figure 2b).

From the Figure 3, it can be seen that in the case of the three extracts for concentrations used 0.5%, 2%, 9%, and 15% the dynamics of the fungus are inversely correlated proportionally with the concentration of the extracts used.

The fungistatic effect of the three extracts can be correlated with the concentration of polyphenols and antioxidant activity. Sage extract with a polyphenol content of 8.46 mg GAE/mL extract inhibits the apical growth of mycelial hyphae and reduces the sporulation percentage to 90% compared to the normal values. At the concentration of 2%, the extracts of European birthwort and celandine did not have an inhibitory effect on both pathogens, compared to the sage extract where the fungistatic effect was visible.

HPLC analysis revealed the presence of rutin and catechin as the main compounds in *S. officinalis* extract in considerably higher concentrations compared to the other extracts. The other two extracts, celandine, and European birthwort extract, present a lower amount of polyphenols, which is why the development of the pathogen at low concentrations of the extracts are close to the control.



(a) (b)

Figure 1. Cultural and morphological features of *Alternaria solani* (a). Colony on PDA agar; (b). Microscopic view of conidia

(a) (b)

Figure 2. Cultural and morphological features of *Fusarium oxysporum* f. sp. *lycopersici*. (a). Colony on PDA agar; (b). Microscopic view of macroconidia

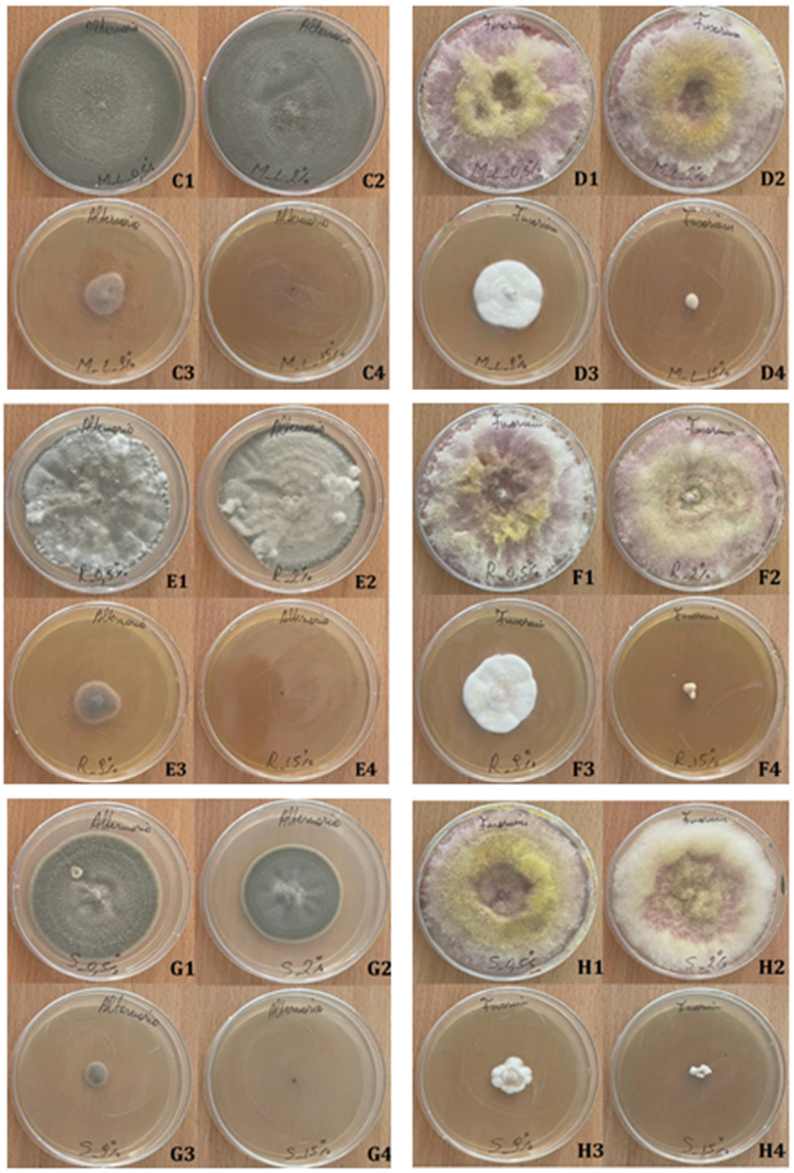
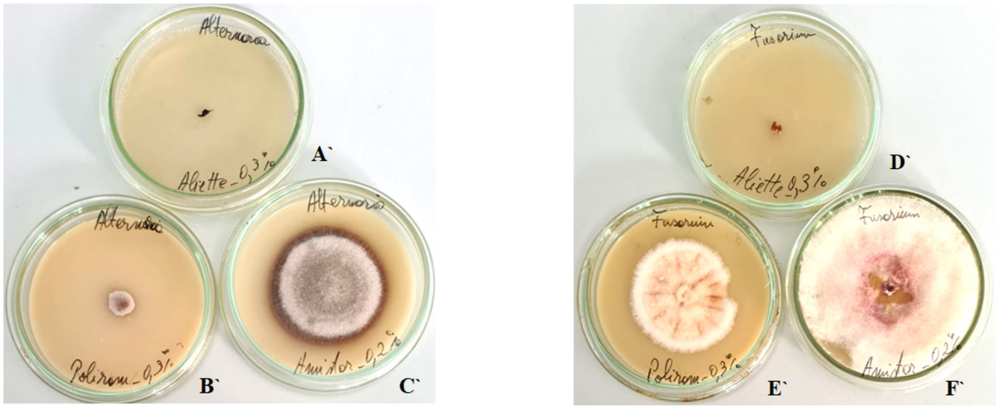


Figure 3. Antifungal effect of european birthwort (C and D), celandine (E and F) and sage (G and H) extracts *in vitro*. *A. solani* series C, E, G and *F. oxysporum* f. sp. *lycopersici:* series D, F, H. C1, D1, E1, F1, G1, H1 = 0.5%; C2, D2, E2, F2, G2, H2 = 2%; C3, D3, E3, F3, G3, H3 = 9%; C4, D4, E4, F4, G4, H4 = 15% extracts.

Evaluation of the fungicidal potential of pesticides in vitro

In our studies, the sensitivity of the two pathogens to the previously presented bioproducts was compared with that to the synthetic pesticides that have aluminum fosetyl, metiram and azoxystrobin as active substances. The concentrations used were 0.3% for aluminum fosetyl (Aliette), 0.2% for azoxystrobin (Amistar), and 0.3% for metiram (Poliram). From the analysis of the Figure 6a (A) it can be observed the extraordinary fungicidal action of aluminum fosetyl on the *Alternaria* mycelium, the percentage of inhibition being 100%. Good results were also observed with the fungicide metiram 0.3% where the inhibition percentage was 88.97% (Figures 4a: B-C).

As in the case of the pathogen *Alternaria*, and against the mycelium of the fungus *Fusarium oxysporum* f.sp. *lycopersici*, aluminum fosetyl had a very good fungicidal activity, the inhibition percentage being 100% (Figure 6b: D'). Azoxystrobin 0.2% had a weak fungistatic effect on the pathogen, the percentage inhibition value being even below that of the three plant extracts. In the scheme, azoxystrobin was shown to have a weak inhibitory action, comparable to variants where bioformulations were used (Figures 4b: B'-C').



(a) (b)

Figure 4. Antifungal effect of fungicides in vitro (a). *A. solani*: (A') aluminum fosetyl 0.3%; (B,') metiram 0.3%; (C") azoxystrobin 0.2%; (b). *F. oxysporum* f. sp. *lycopersici*: (D') aluminum fosetyl 0.3%; (E') metiram 0.3%; (F') azoxystrobin 0.2%

The concentrations used were reported to the effect of the minimum inhibitory concentrations recommended by the phytosanitary codex (aluminum fosetyl 0.2%, azoxystrobin 0.1%, metiram 0.2%).

From the analysis of Figure 5a, strong inverse correlations ($p < 0.001$) were found between percentage inhibition and percentage doses of extract sage used *in vitro* in the study of the development of the pathogen *Alternaria solani*. Significant differences between the experimental variants were observed between the variants treated with extract *S. officinalis* and the rest of the variants in the case of both pathogens.

The same type of association ($p < 0.001$) was also identified in *Fusarium*, but only in the case of variants with extracts (Figure 5b).

From the analysis of Figure 6, strong inverse correlations ($p < 0.001$) were found between percentage inhibition and percentage doses of pesticides used *in vitro* in the study of the dynamics of the pathogen *Alternaria solani*. Significant differences between the experimental variants were observed between the variants treated with fosetyl aluminum and the rest of the variants in the case of both pathogens. The same type of association ($p < 0.001$) was also identified in *Fusarium oxysporum* f. sp. *lycopersici*, but only in the case of variants with synthetic fungicides.

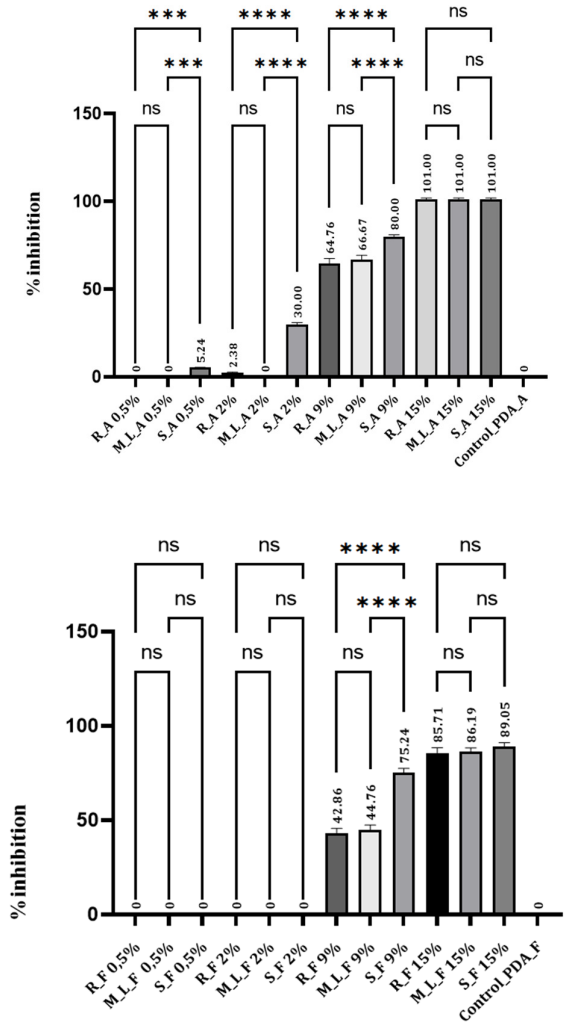


Figure 5. Inhibitory effect of natural extracts

the data are expressed as the mean ± SD values of three independent experiments performed in triplicate, and the values of p were calculated by the one-way ANOVA method followed by Šídák's multiple comparison test.$^{****}p<0.0001$;$^{***}p=0.0003$; $^{ns}p>0.9999, 0.9082, 0.5284, 0.4482, 0.2752, 0.2421$; (a). *A. solani* (b). *F. oxysporum* f. sp. *lycopersici* (R_... - celandine extract; M_L_... - European birthwort extract; S_... – *S. officinalis* extract, PDA – potato-dextrose agar)

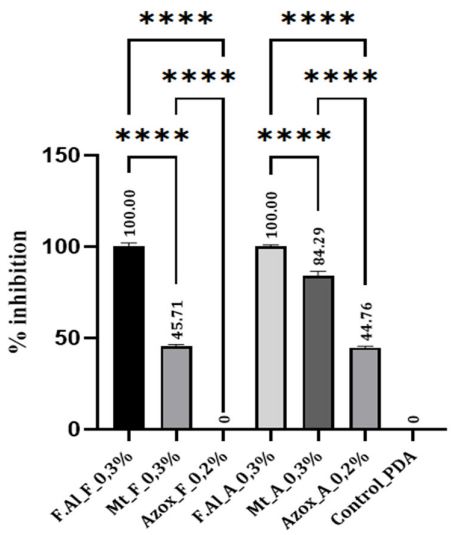


Figure 6. Inhibitory effect of synthetic fungicides
the data are expressed as the mean ± SD values of three independent experiments performed in triplicate, and the values of p were calculated by the one-way ANOVA method followed by Šídák's multiple comparison test. ****p <0.0001, A=*A. solani*, F=*F. oxysporum* f. sp. *lycopersici* (F.Al= aluminum fosetyl; Mt= metiram; Azox= azoxystrobin, PDA – potato-dextrose agar).

Discussion

In our study, the extract obtained by the microwave extraction method from sage presented the highest amount of polyphenols and catechin (8.46 mg GAE/mL extract plant; 446.04 μg/mL extract), while the lowest amount of polyphenols was observed in the extract obtained from *A. clematitis* (5.12 mg GAE/mL extract) and *C. majus* (3.97 mg GAE/mL extract). The effects of antifungal activity of *Salvia* species could be marginally compared to literature data that confirm this as an unexplored field for experimental research. Few scientific studies investigated the ability of the essential oil of *S. officinalis* to inhibit fungal growth, against the toxigenic *Fusarium* sp., several strains related to the crop production and food storage (*Botritis cinerea*, *Verticillium dahliae* and *Penicillium aurantiogriseum*), against *Candida* and other dermatophyte strains (Alexa *et al.*, 2018; Badiee *et al.*, 2012).

Which focused on the species *A. clematitis* L., it was shown that by means of a conventional extraction method and using 60% ethyl alcohol as solvent, the maximum amount of polyphenols recovered from this species was 11.04 mg caffeic acid/g dried plant (Benmehdi *et al.*, 2017). In the case of *Aristolochia bodamae* Dingler, using the Soxhlet extraction method and the same type of solvent mentioned in the previous study, it was possible to obtain three times the amount of polyphenols, namely 31.11 mg GAE/g, compared to the value recorded for *A. clematitis* L. (Benmehdi *et al*., 2017; Ozen *et al.*, 2019).

Nile *et al.* (2021) observed a variation in the total content of polyphenols depending on the parts of the *C. majus* L. plant, which ranged from 7.76 to 23.67 mg/g. The concentration of polyphenols identified in other studies was 17.8 ± 1.59 mg/g DW, this being present in the flowering stage of the species *C. majus*.

The free radical reducing capacity of DPPH in extracts obtained from the plant species also targeted in the present study has been shown in other studies. Thus, the antioxidant activity of the crude methanolic extract obtained from *A. clematitis* L. showed an IC_{50} value of 0.142 mg/mL (Khodabande *et al.*, 2017). Results

of the study by Farid *et al.* (2019) demonstrated that the antioxidant activity of the extract obtained from *C. majus* L. species was 0.41 mg/mL. Also, Oarcea *et al*. (2016), using the same plant species *C. majus* L., found that the antioxidant capacity was 2.399 mM Trolox/ L extract. In addition, the study by Dias *et al.* (2016), a variation in the antioxidant capacity of the extract obtained from *Capsicum baccatum* L. var. *pendulum*, from 0.59 μmol Trolox/g plant material to 2.57 μmol Trolox/g plant material was revealed.

Additionally, it was demonstrated that the extract of garlic significantly reduced the mycelial growth of *A. solani* (Wszelaki and Miller, 2005). Several plant extracts were evaluated and showed antimicrobial activity against plant pathogens under *in vitro* and *in vivo* conditions (Kagale *et al.*, 2004). Essential oils and plant extracts show antifungal activity against a wide range of fungi (Grane and Ahmad, 1988; Wilson *et al.*, 1997; Yousef *et al.*, 2022;). It is known from studies that aqueous extracts of plants viz., *Cymbopogon proxims*, *Allium sativum*, *Carum carvi*, *Eugenia caryophyllus* and *Azadirachta indica* showed strong antifungal activity against fungi such as *Botrytis cinerea*, *Fusarium oxysporum* and *Rhizoctonia solani* (Farkas and Kiraly, 1962).

Many plant diseases are controlled by phenolic compounds (Kue, 1963; Hernández-Ortega *et al.*, 2011). No direct correlation can be established between polyphenol content and antioxidant activity in terms of fungistatic action. This is also confirmed in the specialized literature where it is mentioned that some extracts with a low content of polyphenols had a low antioxidant capacity (on the other hand, others presented a high antioxidant activity and a low polyphenol content (the extracts from the habanero pepper) (Castro-Concha *et al.*, 2012). The action of phenolic compounds on fungi is diversely explained in current studies. Some compounds inhibit cell growth by destabilizing the antioxidant system (Rodriguez-Maturino *et al.*, 2015) or as a result of destroying the integrity of the fungal cell wall (López-Muñoz *et al.*, 2019).

The antifungal action of sage extracts associated with the content of polyphenols on the aggressive pathogens *Fusarium andiyazi* and *Cochliobolus* sp. fungi in asparagus and basil crops was also confirmed (Fan *et al.*, 2010), the percentage of inhibition process being between 55.61% and 82.29%. The fungicidal effect of celandine extract is also mentioned on the pathogens *Fusarium oxysporum* f. sp. *melonis*, *Verticillium dahlia* and *Vermicularia capsica* (Wei *et al.*, 2020). It is interesting to mention the fact that there are taxonomic groups of *Fusarium*, namely *F. culmorum*, *F. graminearum*, *F. solani* and *F. oxysporum* on which the extract of celandine manifests differently an inhibitory effect on the growth of colonies (Gerlach, 1981; Matos *et al.*, 1999). Few data are reported regarding the antifungal action of European birthwort extract on pathogens in cultivated plants. Thus, in Romania, it was shown that the hydroalcoholic extract of European birthwort inhibits the growth of the fungus *Botrytis cinerea* (Miclea *et al.*, 2012). In the international literature, much data on the antifungal effect of the European birthwort extract refer to pathogenic fungi in humans and animals.

The mode of action of fosetyl aluminum is still poorly understood. It seems that this and its main metabolite, phosphonic acid, act both directly, preventing disturbances in the normal functioning of membranes and cell walls, and indirectly, by activating some defense mechanisms of the treated plants (Guest *et al.*, 1994). In this study, azoxystrobin was shown to have a weak inhibitory action, comparable to variants where bioformulations were used.

Our results are confirmed by data from specialized literature. Thus, the contact fungicides mancozeb, metiram, copper oxychloride showed against *Alternaria* sp. an efficacy ranging from 88.51% to 99.98% (Sinha and Prasad, 1989; Hussaini and Singh, 1989). Among the systemic fungicides, pro-piconazole, difenoconazole and azoxystrobin inhibited the growth of mycelial hyphae by up to 99% (Ladumor *et al.*, 2019). Various researches have shown that for the control of *A. solani*, polyram was best rather than captan (Sarkar and Chowdhary, 2004). Experiments on different fungicides *in vitro* observed that hexaconazole and azoxystrobin was very effective against *A. alternata* (Singh and Singh, 2002; Sidlauskiene *et al.*, 2003). Similarly, experience established that Aliette (phosphonate fungicide) combined with another fungicide, mancozeb, enhanced turf quality and controlled what has been referred to as "summer decline of bentgrass" (Lucas, 1995). The mode of

action of phosphonate fungicides is still in argument and obscurity because some scientists consider that these fungicides have direct effect on the fungal pathogen; while others believe on that together a direct effect of pathogenic fungus and a natural host defenses stimulation combine to prevent disease but it was assumed that phosphonate fungicide itself was not directly involved in killing the fungus, rather it was involved in stimulation of the plant's natural physical and chemical defenses against the disease (McDonald *et al.*, 2001).

The celandine and sage extracts showed efficient antifungal effects that provide premises for further experiments and research including consideration of extracts from various parts of the plants. In this direction, the antifungal activities of *Annona muricata* pulp and seed extracts were tested both *in vitro* and *in vivo* for inhibiting *Alternaria alternata* (Fries) Keissler, which is the causative agent of black spots of tomato fruit. It was found that the seed extracts were more efficient than the pulp extracts.

The *in vitro* assay showed a 90% inhibition of *A. alternata* radial mycelial growth by methanol seed extracts, at the highest concentration of 6%. On the other hand, a significant reduction in lesion diameter and disease inhibition by 84% was observed on the tomato fruit treated with methanol seed extracts in the *in vivo* assay (Rizwana *et al.*, 2021).

Conclusions

The use of natural compounds for the control of pathogens is very attractive and represents a new approach in the field of plant protection. The results of the experiments indicated a positive effect of plant extracts, especially sage, on the growth of the two pathogens. Even at low concentration values, the fungistatic effect was strong due to the high content of secondary metabolites (polyphenols and catechin). The antagonistic potential of plant extracts is of interest to be used at large scale in farm operations as these are cheaper than chemicals and generally do not cause environmental pollution or health hazards in man and animals.

The use of resistant varieties is an environmental-friendly method against early blight but resistant varieties are either not available or expensive to develop and require huge investment and technical knowledge. In these circumstances, the current investigation is aimed to develop eco-friendly management methods against early blight. The use of herbal extracts in plant disease management is most significant by being an eco-friendly and cost-effective strategy.

Authors' Contributions

Conceptualization, A.D., C.M. and R.M.; methodology, A.D., C.M., R.M., A.P., D.I.P.(S.), D.V., G.C., I.D.S., D.E.V., I.M.; software, A.D., C.M.; validation, A.D., C.M., R.M., A.P., D.V., G.C.; formal analysis, A.D., C.M., R.M., A.P., D.I.P.(S.), D.V., G.C., D.I.S., D.E.V., I.M.; investigation, A.D., and C.M.; resources, A.D., C.M., R.M., D.I.P.(S.), A.P., D.V., G.C., I.D.S., D.E.V.; data curation, A.D., C.M., R.M.; writing-original draft preparation, A.D., C.M., R.M.; writing-review and editing, A.D., C.M., R.M.; visualization, A.D., C.M., R.M.; supervision, A.D., C.M., R.M. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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