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Efficacy of Antifungal Activities of Some Plant Extracts and Essential Oils against *Macrophomina phaseolina* Strawberry

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ABSTRACT

Macrophomina phaseolina is a significant noxious fungus that spreads through seeds and soil. It is a serious disease agent affecting the strawberry crop (*Fragaria × ananassa* Duch.), commonly known as charcoal rot. Natural plant extracts and their essential oils (EOs) are rich sources of fungitoxic compounds that can serve as alternatives to synthetic fungicides. In this study, *M. phaseolina,* as a fungal plant pathogen, was isolated from infected roots of a strawberry plant. Moreover, the biological effect of three plant extracts: garlic (*Allium sativum*), clove (*Syzygium aromaticum*) and cinnamon (*Cinnamomum zeylanicum*) and their essential oils was investigated against the mycelium growth of *M. phaseolina in vitro*. Plant extracts were extracted using hot and cold extraction procedures and their effects were tested at four concentrations: 25, 50, 75 and 100%. Results revealed that all three investigated plant extracts had a significant inhibitory effect on the mycelium growth (% IMG) of the fungal pathogen at all tested concentrations. Meanwhile, clove had the highest of % IMG, followed by garlic, and cinnamon had the lowest of % IMG (p < 0.0001). The cold extracts of both clove and garlic had a noticeable effect on inhibiting fungal hyphae compared with hot extracts ($p < 0.05$); in contrast, the extraction method had no differential effect on inhibiting fungal hyphae growth, whether the extraction was cold or hot, when cinnamon extracts were used, especially at high concentrations of extracts, 75% and 100% ($p > 0.05$). Moreover, a profound effect of using the EOs of three plants (garlic, clove, and cinnamon) on the mycelial growth of M. phaseolina was observed compared with the control sample ($p < 0.05$). Comparing the use of these oils with that of the fungicide, there were no significant differences in their effect on fungal growth at all tested concentrations ($p > 0.05$) except for the use of garlic oil at a concentration of 25% ($p < 0.0001$). This study suggests that clove, garlic and cinnamon extracts and their EOs could be promising candidates for preventing charcoal rot infection in strawberry; therefore, more *in vivo* studies are needed.

فعالية بعض المستخلصات النباتية وزيوتها العطرية كمضادات فطرية ضد نموفطر *Macrophomina phaseolina* في نبات الفرولة

عبير مسعود القبلاوي[1]، عائشة صالح عامر[1*]، محمد علي عبيد[2]، منى نوري عكريم[2]

فطر *Macrophomina phaseolina* فطر ضار ينتشر عبر البذور والتربة، وهو عامل ممرض خطير يصيب محصول الفراولة(*Fragaria × ananassa* Duch.) ، ويُعرف باسم تعفن الفحمي. تُعد المستخلصات النباتية الطبيعية وزيوتها العطرية مصادر غنية بالمركبات المضادة للفطريات، والتي يمكن أن تُستخدم كبدائل للمبيدات الفطرية الاصطناعية. في هذه الدراسة، تم عزل فطر*M. phaseolina* من جذور نبات فراولة مصابة ومن ثم تم دراسة التأثير البيولوجي لمستخلصات ثلاثة أنواع نباتية وهى الثوم (*Allium sativum*)،والقرنفل (*Syzygium aromaticum*)، والقرفة (*Cinnamomum zeylanicum*) ، كذلك زيوتها العطرية على نمو فطر *M. phaseolina* المعزول في المختبر. أستخدمت طريقتي الإستخلاص على الساخن و وعلى البارد لتحضير المستخلصات النباتية ، وتم إختبار تأثيرها على نمو الخيوط الفطرية بأربعة تراكيز وهى 25%، 50%، 75%، و100%. أظهرت النتائج أن جميع المستخلصات النباتية للانواع النباتية الثلاثة كان لها تأثير مثبط كبير على نمو الفطريات الممرضة وذلك بنسبة تثبيط عالية عند جميع التركيزات المختبرة. في حين سجل

القرنفل أعلى نسبة تثبيط، يليه الثوم، وسجل القرفة أدنى نسبة (قيمة الاحتمالية $p < 0.0001$). كان للمستخلصات الباردة لكل من القرنفل والثوم تأثير ذو دلالة إحصائية في تثبيط نمو الخيوط الفطرية مقارنةً بالمستخلصات الساخنة (قيمة الاحتمالية $p < 0.05$). في المقابل، لم يكن لطريقة الاستخلاص أي تأثير مختلف على تثبيط نمو الخيوط الفطرية، سواءً كانت باردة أو ساخنة، عند استخدام مستخلصات القرفة، خاصةً عند التركيزات العالية، 75% و100% (قيمة الاحتمالية $p > 0.05$). علاوة على ذلك، لوحظ تأثير كبير لاستخدام الزيوت العطرية للنباتات الثلاثة (الثوم والقرنفل والقرفة) على نمو فطر *M. phaseolina* مقارنةً بعينة التحكم (قيمة الاحتمالية $p < 0.05$). بمقارنة استخدام هذه الزيوت مع استخدام المبيد فطري، لم تُلاحظ فروقٌ ذات دلالة إحصائية في تأثيرها على نمو الفطرعند جميع التركيزات المختبرة (قيمة الإحتمالية $p > 0.05$)، باستثناء استخدام زيت الثوم بتركيز 25% (قيمة الاحتمالية $p < 0.0001$). تشير هذه الدراسة إلى أن مستخلصات القرنفل والثوم والقرفة وزيوتها العطرية قد تكون مرشحةً واعدةً للوقاية من عدوى تعفن الفحمي في الفراولة؛ لذا، يلزم إجراء المزيد من الدراسات الحيوية.

INTRODUCTION

Strawberry (*Fragaria* × *ananassa* Duch.) is a horticultural crop belonging to the Rosaceae family and the genus *Fragaria*. It is a highly nutritious fruit crop with significant global economic value (Tane, 2022). The world produces almost three million tons of this fruit, which is grown on enormous tracts of land—roughly 372,000 hectares (Fao, 2018). The rise in strawberry yields over the last ten years may have been caused by scientific advancements in strawberry production, better cultivation methods, and the control of pests and diseases (Lee, 2014; Pastrana *et al*., 2016). However, due to their extreme tenderness, susceptibility to mechanical damage, increased respiration, and high susceptibility to fungal diseases like Fusarium wilt, charcoal rot, root rot, and crown rot, strawberry production is highly vulnerable to field and post-harvest losses (Demirer-Durak and Demirci, 2018; Kesimci *et al*., 2022). *Macrophomina phaseolina* is one of the most well-known soil fungi that causes strawberry charcoal rot. It has a prolonged life and an extremely competitive saprophytic capacity (Ijaz *et al*., 2013).

One of the most destructive strawberry diseases worldwide, *M. phaseolina* is a fungal pathogen that infects over 500 plant species. (Kaur *et al*., 2012; Sánchez *et al*., 2013; Hajlaoui *et al*., 2015). It is distinguished by the browning of cortical and vascular root tissues. (Koike, 2008). Controlling *M. phaseolina*-produced microsclerotia is extremely challenging. They are usually the primary form of inoculum for future infections and allow *M. phaseola* to survive over the winter because of their heat-tolerant and resistant structure (Tajdinian *et al*., 2022). According to Baggio *et al*. (2019), strawberry charcoal rot damage in the Southeast US is estimated at 15-20 % of the annual yield loss, or $3-4 million, and also destroys 2-5 % of strawberry plants in Florida every year and 80 % of fields when weather conditions are favourable. Furthermore, the crowns and roots of strawberry plants in Iran were found to contain *M. phaseolina*, which resembles wilting and rotting symptoms. (Tajdinian *et al*., 2022).

If chemical fungicides are applied excessively and for an extended period of time, they may cause ecosystem instability and the emergence of resistant fungal strains (Lucas *et al*., 2015). Research is increasingly highlighting the potential of plant-derived natural chemicals as a safer and more sustainable alternative to synthetic fungicides. These botanical extracts provide a potential source of biological substances that are easily degradable, systemic, and non-toxic for the treatment of fungal diseases (Ahmadi *et al*., 2022; Gizaw *et al*., 2022). These extracts' bioactive compounds have been demonstrated in studies to either directly stop these fungal pathogens from growing (Ahmadi *et al*., 2022) or to elicit a host defence response, thereby reducing the severity of the disease (Schneider and Ullrich, 1994; Zhou *et al*., 2023; Abbod *et al*., 2025). Biological or natural inducers can be used to stimulate the internal resistance mechanisms of plants (Attia *et al*., 2016; Attia *et al*., 2022).

Antioxidant, phenolic and antimicrobial oils containing aromatic polyethene (PE) are effective against phytopathogenic fungi and significantly improve plant health (Veloz-García *et al*., 2010; Raveau *et al*., 2020). It has recently been used to boost plant production and improve plant growth barriers because the plant-stimulating compounds in the vegetable oil emulsion give it a very high efficiency and no harm to the environment or human health (Yang *et a*l., 2022; Hashem *et al*., 2023).

Increasing research suggests that essential oils (EOs) are viable alternatives to fungal growth inhibitors. Plants accumulate secondary aromatic compounds that have antifungal or antibacterial qualities (Gurjar *et al*., 2012). Considering that compounds found in nature may be able to treat fungal diseases (Dayan *et al*., 2009; Antunes and Cavaco, 2010; Abbey *et al*., 2019). As an illustration, Groppo *et al*. (2007) found that fresh garlic has a potent antibacterial effect on oral streptococci, whereas essential oils and cinnamon extracts (*Cinnamomum cassia* L.) have been studied for their many therapeutic applications in antiseptic products against pathogenic fungi (Absalan *et al*., 2012; Soliman *et al*., 2015).

Traditional medicine also uses clove (*Syzygium aromaticum* L.) for its broad-spectrum antifungal and antibacterial properties (Daniel *et al*., 2015). Rahhal (1997) showed that *Alternaria tenuis*, *Sclerotinia sclerotiorum*, *Fusarium solani*, and *Rhizoctonia solani* all had their mycelial development totally suppressed by clove oil, with minimal sclerotial development and a maximum inhibition (77.65%) of mycelial growth of *M. phaseolina*.

In addition, phytoextracts of garlic cloves and neem (15 and 20%) were reported to achieve maximum mycelial growth inhibition (92.46 and 100%) in *M. phaseolina*, respectively (Khaire *et al*., 2018).

Moreover, extracts of rosemary (*Rosmarinus officinalis*), clove (*Syzygium aromaticum*), cinnamon (*Cinnamomum zeylanicum*), and garlic (*Allium sativum*) have antifungal potential against the pathogen *M. phaseolina* (Lorenzetti *et al*., 2018). The aim of this study was therefore to investigate the antifungal activity of the aqueous extracts of selective plants (garlic, cinnamon, and clove) and their EOs against *M. phaseolina*, which infects strawberries, and to compare their effectiveness with that of the fungicide Mancozeb using *in vitro* assays.

MATERIALS AND METHODS

Isolation, purification and identification of the fungus

One hundred plant samples of strawberry plants (festival variety) exhibiting clear symptoms of charcoal rot were collected from strawberry farms in the Wadi Al Rabie city, southeast of Tripoli, Libya. The plant samples were placed separately in sterile plastic bags and then immediately transported to the Plant Protection Department, Faculty of Agriculture, University of Tripoli. Once the samples arrived at the laboratory, the pathogen *M. phaseolina* with varying degrees of pathogenicity was isolated from the roots of infected strawberry plants as follows: infected roots were washed with tap water, then cut into 3-cm-long pieces. The surface of each piece was then sterilised with 70% ethyl alcohol for a minute and dried on sterile filter paper. After that, the root pieces were washed several times with sterile water and dried again on sterile filter paper. Five root fragments were then placed in each Petri dish containing sterilised Potato Dextrose Agar (PDA) and autoclaved at 15 lbs pressure (121°C) for 20 minutes. The dishes were then incubated at 25 ± 2°C for 4–5 days (Atia and Esh, 2005). The established mycelium was collected and transferred onto fresh PDA medium. The hyphal tip approach was then used to purify each isolated fungus (Madan and Thind, 1998). The isolated fungi were identified based on the colony phenotype, the shape and colour of the spores and the shape of the sclerotia it forms (Gilman, 1957; Domsch *et al*., 1980). For additional research, the purified isolates were stored in a refrigerator at 5 ºC on (PDA) slants.

Preparation of plant extract

The plant components were extracted using both cold and hot methods. Three targeted plants, garlic cloves, cinnamon, and cloves were purchased from herbalist shops. Plant details are depicted in Table 1. They were washed thoroughly with tap water to remove debris and impurities, then rinsed with distilled water. The garlic, cinnamon, and cloves were dried in the shade until all moisture was removed. Two extraction methods were used: hot and cold. In the first method, 20 g of each plant was weighed after being ground with an electric blender and then mixed with 100 mL of distilled water. The mixture was then heated to the boiling point and cooled. Afterwards, the mixture was filtered through a funnel containing Whatman No. 1 filter paper, then through a 0.22µm bacterial filter (13 mm Syringe Filter, 0.22 µm, PTFE, 100-pk, Restek). For the cold extraction method, 20 g of each ground plant was soaked in 100 mL of sterile distilled water for 24 hours. The following day, the extract was filtered as described above. The extract was obtained at a concentration of 20%. The extracts were prepared at four concentrations, 25, 50, 75, 100% for each extract, hot and cold (Fawzi *et al*., 2009).

Table 1. Plant types used in plant extract preparation

Extracted part of the Plant	Family	The scientific name of the Plant	Common name
Garlic cloves	Amaryllidaceae	*Allium sativum*	Garlic
Dried flower buds	Myrtaceae	*Syzygium aromaticum*	Clove
Rhytidome	Lauraceae	*Cinnamomum zeylanicum*	Cinnamon

Essential Oils

Commercial EOs of Garlic, cinnamon and clove were used in the study, purchased from Pharmacies in Tripoli, Libya.

In vitro antifungal activity of plant extracts against the mycelial growth of *M. phaseolina*

Four different concentrations of each plant extract were utilised: 25%, 50%, 75%, and 100%. To evaluate the influence of garlic, clove and cinnamon plant extracts (shown in Table 1) on the growth of *M. phaseolina*, one 6 mm diameter disc from active cultures was placed into each of four Petri dishes containing PDA medium along with the plant extract. The experiment included a negative control set up with sterile distilled water and a positive control created using the fungicide mancozeb (ethylene bisdithiocarbamate), which was made following the manufacturer's guidelines by combining 0.5 grams into 100 ml of sterile distilled water. Mancozeb served as a broad-spectrum fungicide that prevents fungal growth through contact. Three replicate plates with plant extract agar per isolate were incubated at 25±2°C. The inhibition of mycelial growth is calculated by expressing the fungus growth on the plant extract agar as a percentage of the growth on the PDA, using the formula:

$$\%MGI = DC - DT / DC \ X \ 100$$

where %MGI represents the percentage inhibition of mycelial growth, DC denotes the control diameter, and DT denotes the test diameter. The effectiveness of the extracts in inhibiting growth was assessed using the scale established by Sangoyomi (2004).

In vitro screening for antifungal activity of EOs on *Macrophmina phaseolina* growth

Antifungal tests were performed *in vitro* in accordance with the method described by Pitarokili *et al*. (2003), using 9-cm Petri dishes containing potato dextrose agar (PDA). Tween 20 (0.05) was used to dissolve the essential oils (garlic, cinnamon, and clove) in water, which were then added to the PDA right before it was poured into the Petri dishes at 45-50°C. The concentrations that were tested were 25, 50, 75, and 100%. The same amount of Tween 20, combined with PDA, served as the control. As soon as the Petri dishes were ready, a sterile corkborer was used to cut out a 6 mm diameter test-type disc from the edge of the active cultures on the PDA plates and place it in the centre of each fresh PDA plate. Petri dishes were incubated in darkness at 24°C. The average growth rate of three replicates of each fungal species was calculated after five days, or until the control fungi had filled the petri dishes. To record the mycelial growth, the colonies' diameters were measured in opposing directions on a daily basis. By averaging both dimensions every day, we were able to calculate the percentage of mycelial growth inhibition. As directed by the manufacturer, 0.5–5 g of ethylene bis-bisdithiocarbamate (mancozeb) was mixed with 100 ml of sterile distilled water to create the positive control, while the negative control was set up in sterile distilled water. Mycelial growth inhibition is defined as the fungus's growth on agar of the plant extract expressed as follows:

$$\%MGI = DC – DT / DC \ X \ 100$$

where MGI = % inhibition of mycelial growth, DC = control diameter, and DT = test diameter. A scale outlined by Sangoyomi (2004) was used to assess the extracts' inhibitory effect.

Statistical analysis

The data were displayed as mean ± SD from three replicates at each concentration after statistical analysis using ANOVA in GraphPad Prism 9 to assess the most effective *in vitro* treatments among plant extracts, natural oils, and fungicides. Mean values were compared at the last significant level of difference, 5%, using Tukey's multiple comparisons test.

RESULTS AND DISCUSSION

Antifungal properties of plant extracts against the mycelium growth of *M. phaseolina* in an *in vitro* experiment.

Four fungal pathogens of *M. phaseolina* with varying pathogenicity were isolated from infected roots of a strawberry plant in this study, and the most virulent one was used. The effect of plant extracts, namely, garlic (*A. sativum*), clove (*S. aromaticum*), and cinnamon (*C. zeylanicum*) that were extracted under two different extraction conditions, hot and cold, and their essential oils were tested for their capacity to inhibit *M. phaseolina* growth on PDA at four concentrations (25, 50, 75, and 100%). The plant extracts of three tested plant species showed inhibition of mycelial growth compared to the control. However, inhibition ratios varied with the extraction method (hot or cold) and extract concentration (**Figures 1, 2, and 3**). The garlic extract using either of the two extraction methods, hot and cold, had a statistically significant effect on the mycelium growth at all tested concentrations compared with the negative control ($p < 0.0001$), where the higher mean %MGI of *M. phaseolina* was observed in cold garlic extract at concentrations of 75% and 100% (100%), followed by 50% cold garlic extract (92.93%) and 25% cold garlic extract (88.87%). The lowest mycelial suspension of 13.83 % was recorded in 25% hot garlic extract (**Figure 4**). The same antifungal activity was observed when comparing the effect of the fungicide (positive control) with that of hot garlic extracts at all concentrations ($p < 0.0001$). However, when comparing the effects of cold-extracted garlic extracts and the fungicide, there was only a statistically significant difference when the 25% concentration of garlic extract was applied ($p = 0.0002$). It was also found that there were statistically significant differences between the different concentrations of garlic extracts when used as antifungal agents ($p < 0.0001$). The interesting result was that there were significant differences between the effects of hot and cold extracts on fungal growth, even when the same concentration was applied ($p < 0.0001$), showing a greater antifungal activity of cold garlic extracts (**Figure 1**).

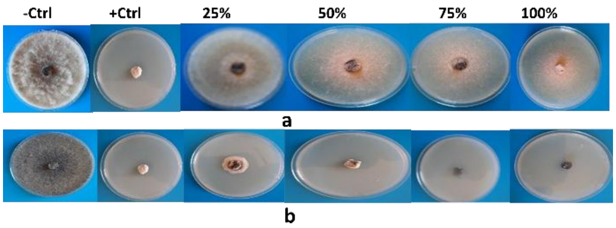

Figure 1. The antifungal effect of different concentrations of garlic extracts (25, 50, 75 and 100%) on the *in vitro* mycelium growth of *M. phaseolina* after 5 days of incubation on PDA at 25 °C. (a) hot extraction, (b) cold extraction. Negative control, without garlic extract (- Ctrl) and positive control, fungicide (+ Ctrl).

Moreover, there were statistically significant differences when comparing the effects of different concentrations of hot extracts ($p < 0.0001$, 0.0026 and 0.0016). However, the differences in the effects of different concentrations of cold garlic extracts as a fungicidal agent were not statistically significant ($p > 0.05$), as there was complete inhibition of the growth of the fungus *M. phaseolina* after 5 days of incubation. This result indicates the greater effect of the cold garlic extracts on fungal growth. The strong toxic properties of garlic extract against several fungal pathogens are likely because garlic contains organosulfur compounds such as allicin, which is a thiosulfate that contains two allyl groups as carbon chains, which in turn reacts with low molecular weight cellular thiols such as glutathione, converting its state to a more oxidised state, causing apoptosis (Carreón-Delgado *et al*., 2023). This finding is consistent with that of Lakhran and Ahir (2020), who found that, among six plant extracts tested as eco-friendly fungicides against *M. phaseolina*, garlic extract was the most effective at reducing root rot incidence in the chickpea crop. This also accords with Hingole and Kendre (2021) observations, which showed that among nine tested plant extracts, the highest inhibition of *M. phaseolina* fungus growth (79.36%) was recorded with garlic extract.

Prior studies have noted the importance of evaluating the efficacy of plant extracts as biocontrol agents and alternatives to synthetic fungicides for managing fungal infections that cause root rot in strawberry (Ruiz-Romero *et al*., 2018). In this study, similar results to those obtained when garlic extracts were used as an antifungal against *M. phaseolina* were also observed with clove extracts as a bio-antifungal agent, whether using hot or cold extraction techniques, across all tested concentrations, compared to the control sample ($p < 0.0001$) (**Figures 2 and 4**).

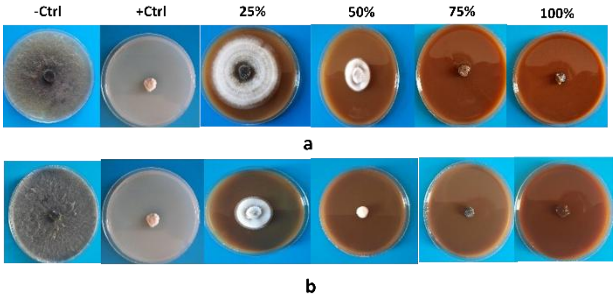

Figure 2. The antifungal effect of different concentrations of clove extracts (25, 50, 75 and 100%) on the *in vitro* mycelium growth of *M. phaseolina* after 5 days of incubation on PDA at 25 °C. (a) hot extraction, (b) cold extraction. Negative control, without clove extract (- Ctrl) and positive control, fungicide (+ Ctrl).

Among the different concentrations tested, the maximum mycelial inhibition of 100% was observed at the 50%, 75%, and 100% concentrations of cold extracts and was significantly superior to the 25% concentration (86.23%). When comparing the efficacy of clove extracts with that of a fungicide (positive control), a highly statistically significant difference was observed when low concentrations of clove extracts (25%) of both hot and cold extracts ($p < 0.0001$) and $p = 0.0027$ with medium concentrations (50%) of the hot extracts were applied. This inhibitory effect of clove extracts increased with increasing extract concentration, as no significant differences were found between the use of hot and cold clove extracts and the use of fungicide at concentrations of 50% for cold extract, 75% and 100% for both extraction methods, hot and cold ($p > 0.05$). These results appear to be due to clove extracts having antioxidant and antimicrobial properties, as they contain phenolic compounds, such as eugenol, flavonoids, hydroxybenzoic acids, and hydroxyphenyl propene (Yassin *et al*., 2020). Pina-Vaz *et al*. (2004) found that carvacrol, present in cloves, is able to kill *Candida albicans* by causing damage to the plasma membrane, leading to the death of the organism as a result of leakage of the plasma membrane contents. This suppressive effect of the cold clove extract, which was more pronounced than the hot extract, on the fungus *M. phaseolina* was consistent with the results obtained using cold and hot garlic extracts. A possible explanation for this might be that Hot extraction may have resulted in the loss of some of the antifungal properties of the extract that were retained during cold extraction.

More *in vitro* evaluation of the use of bioagents as a suppressor of the mycelium growth of *M. phaseolina* was conducted using cinnamon extracts. The effectiveness of cinnamon extracts against the pathogen was noticeable (**Figure 3**).

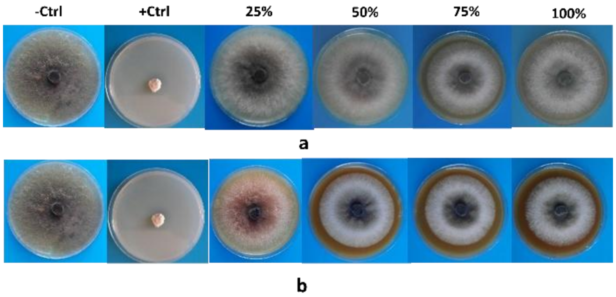

Figure 3. The antifungal effect of different concentrations of cinnamon extracts (25, 50, 75 and 100%) on the *in vitro* mycelium growth of *M. phaseolina* after 5 days of incubation on PDA at 25 °C. (a) hot extraction, (b) cold extraction. Negative control without cinnamon extract (- Ctrl) and positive control, fungicide (+ Ctrl).

There was a significant difference in the % MGI of *M. phaseolina* using different concentrations of cinnamon extract and two extraction techniques, hot and cold, compared with the control sample and fungicide ($p < 0.0001$) **(Figure 4)**. Maximum %MGI of 51.43 was noticed with 100% cold cinnamon extract, followed by 100% hot cinnamon extract (41.47%) and 75% cold cinnamon extract (41.07%). In contrast to garlic and clove results, however, the lowest %MGI of 12.53% was recorded with 25% hot cinnamon extract. This finding broadly supports the work of Lorenzetti *et al.* (2018), who showed that the extracts of rosemary, garlic and clove have antifungal potential for the pathogen *M. phaseolina*; in contrast, cinnamon extract has the lowest biological effect on its mycelium. Cinnamonaldehyde, eugenol, and linalool are the primary bioactive substances found in cinnamon bark. These substances exhibit a wide range of antibacterial action and are neither poisonous nor carcinogenic to mammals (Ojaghian *et al*., 2014). Bang *et al.* (2000) report that trans-cinnamaldehyde exhibits antibacterial activity against Saccharomyces cerevisiae by inhibiting the wall-synthesising enzymes chitin synthase I and β-(1,3)-glucan synthase. As a result, it might be a component of the human diet or utilised as a preservative.

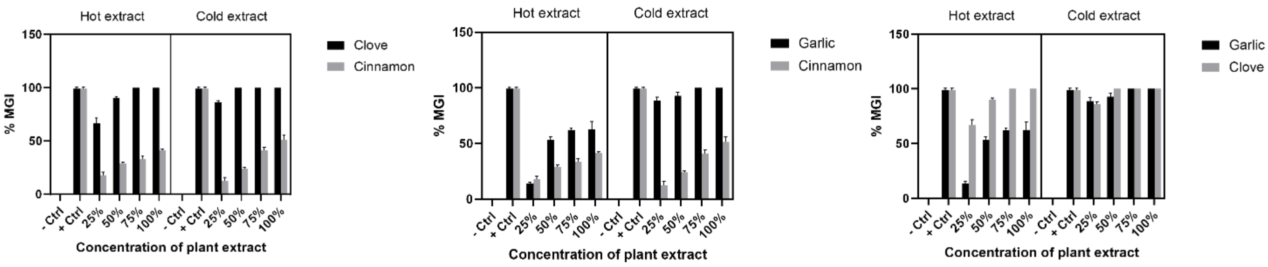

Figure 4. Effect of different concentrations of three plant extracts, garlic, clove and cinnamon extracted under cold and hot conditions on the mycelium growth of *M. phaseolina*.

Since chemical products can be hazardous to both humans and the environment, bio-fungicides derived from plants may be used to biocontrol fungal infections. When comparing the effects of the three plant extracts at the tested concentrations, clove had the strongest inhibitory effect on fungal hyphal growth, followed by garlic, and then cinnamon, which had the least effect ($p < 0.0001$). Moreover, the cold extracts of both clove and garlic had a noticeable effect on inhibiting fungal hyphae compared with hot extracts ($p < 0.05$); in contrast, the extraction method had no differential effect on inhibiting fungal hyphae growth, whether the extraction was cold or hot, when cinnamon extracts were used, especially at high concentrations of extracts, 75% and 100% ($p > 0.05$) (**Figure 4**). These findings corroborate those of Šernaitė *et al*. (2020), who found that both clove and cinnamon extracts were equally efficient in inhibiting *Botrytis cinerea* on Petri plates and that clove extract at 12 mL L^{-1} was beneficial in decreasing grey mould infection on detached strawberry leaves. Nevertheless, the findings of the detached strawberry leaf assay demonstrated that clove extract was a more successful biocontrol agent.

Antifungal properties of EOs against the mycelium growth of *M. phaseolina* in an *in vitro* experiment.

In this study, a profound effect of using the essential oils of three plants (garlic, clove and cinnamon) on the mycelium growth of *M. phaseolina* compared with the control sample was observed ($p < 0.05$) (**Figure 5**). Comparing the use of these oils with that of the fungicide, there were no significant differences in their effect on fungal growth at all tested concentrations ($p > 0.05$) except for the use of garlic oil at a concentration of 25%) ($p < 0.0001$) (**Figures 6 and 7**). In addition, there were no significant differences among the tested concentrations of garlic oil, except for 25% ($p < 0.0001$), which had the lowest %MGI of 34.43%. There were also no significant differences between the ESO of clove and cinnamon at all tested concentrations ($p > 0.05$) (**Figures 6 and 7**).

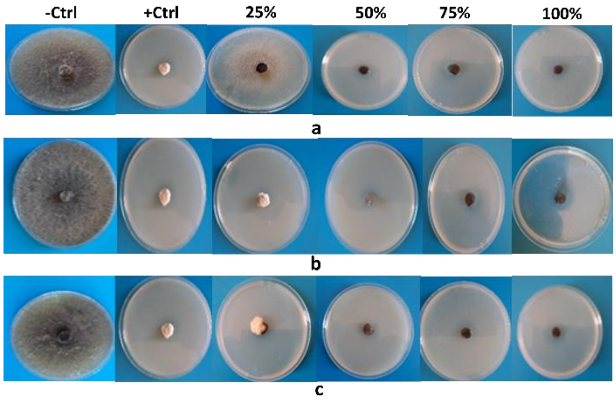

Figure 5. The antifungal effect of different concentrations of EOs (25, 50, 75 and 100%) against the *in vitro* growth of *M. phaseolina* after 5 days of incubation on PDA at 25 °C. (a) EOs of garlic, (b) EOs of clove and (c) EOs of cinnamon. Negative control (-Ctrl) and positive control (+ Ctrl).

As shown in Figure 6, the use of EOs had a greater effect than the use of hot-extracted plant as an antifungal agent. There were significant differences in the %MGI between the use of garlic oil and hot garlic extract ($P < 0.0001$) at all tested concentrations. However, these differences were diminished when comparing the effects of different concentrations of EOs of garlic with cold garlic extracts ($p = 0.0024$) (**Figure 7**), which suggests that cold extract has a stronger inhibitory effect on the growth of *M.phaseolina* than hot extract. Regarding cinnamon extracts, there were significant differences between the %MGI cinnamon essential oil and those of cinnamon extracts (hot and cold) using different tested concentrations ($p < 0.0001$). As expected, there were no significant differences when comparing the inhibition effect between cold clove extract and clove oil ($p > 0.05$), except at the low concentration of the cold extract (25%) ($p < 0.0001$); however, this difference was reversed when comparing hot extracts of clove with its essential oils, as the significant differences disappeared at high concentrations (75% and 100%) of the hot clove extract ($p > 0.05$).

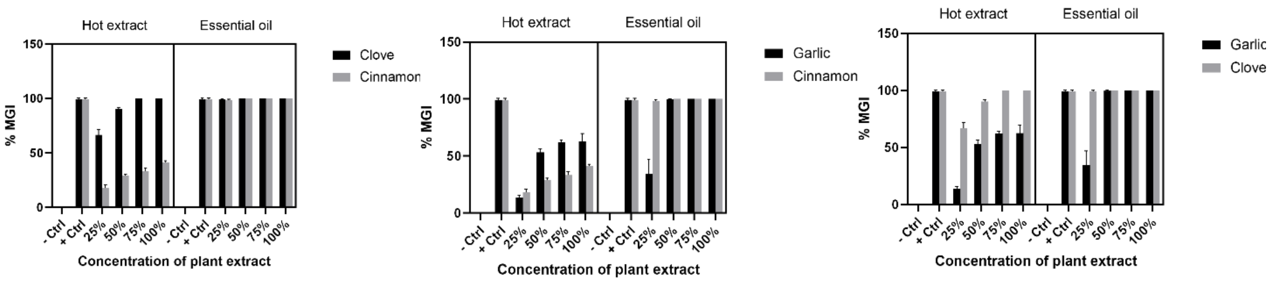

Figure 6. Comparison of the efficacy of three hot plant extracts, garlic, clove and cinnamon and their essential oils at different concentrations (25, 50, 75 and 100%) against the mycelium growth of *M.phaseolina*.

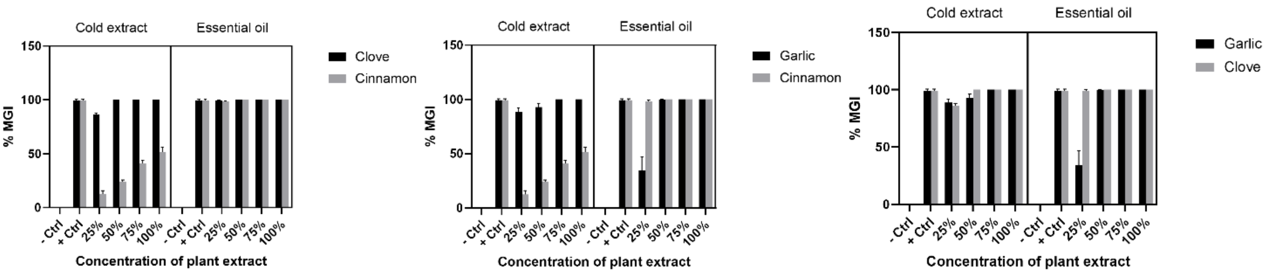

Figure 7. Comparison of the efficacy of three cold plant extracts, garlic, clove and cinnamon and their essential oils at different concentrations (25, 50, 75 and 100%) against the mycelium growth of *M. phaseolina*.

It is encouraging to compare our findings with those of previous studies, which reported that the use of EOs can suppress the growth of *M. phaseolina*. For example, Beg and Ahmad (2002) indicated that clove oil had a high inhibition rate of *Fusarium chlamydosporum* and *M. phaseolina*. Furthermore, when comparing the effectiveness of three EOs: clove, mint, and garlic on the growth of *M. phaseolina* fungus, clove oil was found to be the most effective in inhibiting fungal growth. This oil reduced fungal growth by 69.81%, 100%, and 100% when used at concentrations of 5%, 10%, and 15%, respectively. Mint oil came in second, and garlic oil in third place in terms of effectiveness (Selim *et al*., 2021). In addition, the antifungal effect of EOs of basil, tea tree, citronella, mint, thyme, Ginger, sweet Orange, cinnamon and clove on the mycelial growth of *M. phaseolin*a was evaluated *in vitro* at doses of 0.8, 1.6, 3.2, 6.4 and 12.8 µL. The findings demonstrated that clove, oregano, and cinnamon essential oils completely inhibited mycelial development (de Figueiredoda *et al*., 2023). Based on our results, it is therefore recommended to conduct *in vivo* studies on the use of the tested plant extracts and their EOs to control charcoal rot in strawberries.

CONCLUSION

This study confirmed that the extracts of three tested plants, clove, garlic and cinnamon had an inhibitory effect against mycelium growth of *M. phaseolina* under *in vitro* conditions. Our study also indicates that the strength of this effect may depend on the extraction method, whether it was hot or cold. Cold extracts were found to have a stronger inhibitory effect than hot extracts, with clove extract exhibiting the greatest effect, followed by garlic, while cinnamon extract had the least effect among the three plant extracts. The research has also shown that the natural oils of these plants had a stronger suppressive effect on the mycelium growth of *M. phaseolina* than their extracts. Based on the findings of this study, it has been suggested that further *in vivo* studies of clove, garlic and cinnamon extracts and their natural oils should be conducted to develop their biocontrol properties.

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