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The Effect of Some Plant Extracts on Pathogenic Fungi Isolated from Greenhouses

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Abstract The development of fungal infections and their spread through leaves, stem and soil in greenhouses for agricultural crops is a threat to many diverse agricultural crops due to the environmental conditions they provide, resulting in economic losses for both farmers and the country. In the current laboratory study, the types of fungi were isolated and diagnosed for a group of different plants in the plastic house, namely (pepper, cucumber, chard, spinach, eggplant, strawberry). After isolation and diagnosis, the types of fungi (*Penicillium*, *Fusarium*, *Aspergillus*, *Alternaria*, *Rhizopus*) were obtained, as they were the reason for the appearance of dark spots in the leaves of the plants under study. *Alternaria* spp. and *Fusarium* spp. were used to test plant extracts on them and demonstrate their inhibitory abilities. Three types of aqueous extracts were tested separately: cloves (*Dianthus caryophllus*), cinnamon (*Cinnamomum verum*) and ginger (*Zingiber officinale*) at two concentrations 25, 50% as a first step. Then, in the second step, the most inhibitory extract was mixed, which had a 100% inhibition rate, which is cloves and tested on two fungi (*Alternaria*, *Fusarium*) at the same concentrations 25, 50% with the cinnamon once and with ginger again, the process of mixing two types of extracts gave equal inhibitory results, which are 100% For both types of fungi, unlike using only one type of extract, the result was varying inhibition of fungal cultures. This opens the door to the possibility of using types of plant extracts and mixing them together to combat agricultural pests, as they contain active substances in varying proportions. When combined, they provide more inhibitory antibodies to pathogenic fungi and are used as environmentally friendly biocides.

Key Words *Alternaria*, Plant Extracts, Ginger, *Dianthus caryophllus*, Pathogenic Fungi

INTRODUCTION

Agricultural crops in greenhouses are the most intensive form of agricultural production, where ideal climatic conditions of temperature and humidity are available for plant growth, it is also vulnerable to the development of many fungal diseases that affect it and are transmitted through the leaves, stem and soil [1], as diseases are a major factor in the decline in agricultural yields, which leads to huge economic losses [2]. Since 1940, chemical and industrial fungicides have been used mainly to combat plant pathogens. Recent research has shown that the number of plant-pathogenic fungi is constantly increasing, as this increase leads to losses in agricultural products all over the world In addition to the negative effects caused by industrial pesticides on the environment, humans and animals, in addition to the resistance of fungi to these pesticides, all these reasons prompted scientists and researchers to search for new strategies to combat fungi [3]. The strategy is to implement a method that is safe, applicable and costs effectively to manage field diseases in the future, the use of plant extracts in agriculture is a suitable alternative method in the plant disease control program and they can act as major antifungals without leaving any toxic residues on the agricultural product [4].

Natural plant extracts regulate plant growth and participate in the defensive response, limiting the growth of pathogens. Various experimental conditions have led to the discovery of antifungal activity in aqueous extracts and organic solvents from plants of different origins [5]. Plant extracts are a source of active ingredients such as terpenoids, phenols, alkaloids, flavonoids, soaps, essential oils and others that are low in toxicity to crops, environmentally friendly and effective for fungal growth. According to previous studies, plant extracts have been used against a wide range of plant pathogenic fungi such as *Alternaria solani*, *Aspergillus fumigatus*, *A. niger*, *A. flavus*, *A. fumigatus*, *Fusarium solani*, *F. oxysporum*, *Bipolaris orzyae*, *Botrytis cinerea*, *Curvularia lunata* and *F. verticilliodies* [6]. The aim of the current study

was to isolate the most important fungi causing disease from plant in some greenhouses in Diyala Governorate and to study the effect of treatment with water extracts of some plants towards these fungi.

METHODS

Isolation and Diagnosis of Fungi

To identify disease-causing fungi in plants grown in some nurseries in Baqubah, the capital of Diyala Governorate, which included six plants: eggplant, pepper, cucumber, strawberry, chard and spinach, three samples were taken from each plant, bringing the total number of samples to 18. These samples included leaves showing some disease symptoms. The collection process took place during January and February 2025. The samples were placed in plastic bags labeled with all important information, including the plant type, the nature of the symptoms and the time of collection. The bags were then transferred to the Mycology Laboratory in the Department of Life Sciences, College of Education for Pure Sciences, University of Diyala, for fungal diagnosis.

After collecting the samples represented by plant leaves washing them with distilled water, sterilizing them with diluted alcohol and water and drying them on dry paper, the infected leaves were cut to a length of 1 cm under sterile conditions. The pieces of dry leaves were planted on a medium Potato Dextrose Agar medium (PDA), then the dishes were incubated at a temperature 25±2 for 3-7 days or until the fungal growths appear. After the fungal cultures grew, a sample was taken from each fungal culture and examined microscopically to determine the characteristics of the conides and to identify the fungal species.

The percentage of fungal isolates was calculated according to the following equation:

$$\text{Percentage of appearance} = \text{N. of isolates of the genus or species/total N. of isolates} \times 100$$

Preparation of Plant Extracts

In order to test the effect of aqueous extract of some plants on fungi isolated from nursery plants, aqueous extracts of ginger, cloves and cinnamon were prepared, which were obtained from herbalists' shops in the local market of Baquba city. Aqueous plant extracts were prepared by the method described by Ismail [7]. The plant samples were washed with distilled water, then the ginger samples were cut and left to dry for two weeks, while the cinnamon and cloves were washed, sterilized and left to dry. Each sample was then ground separately using a sterile grinder. Then weigh 100 grams of each ground plant material in 100 mL of distilled water (1:1 weight/volume) for 3 minutes and beat with a blender. The resulting extract was filtered using medical gauze in double layers to remove plant material residues. Then filter the extract again using filter paper. Place the centrifugally filtered extract at 4000 rpm for 5 minutes to obtain a homogeneous aqueous solution. The upper liquid of each solution was then filtered through a 0.45 µm diameter membrane filter to avoid any microbial contamination. Then the concentrations 25, 50 mL were prepared and stored in a dark glass container at a temperature of 5°C in the refrigerator until use.

PH Measurement

Weigh (1 g) of plant powder for each of the plants (cloves, ginger, students) and mix each type with (5 mL) of distilled water and leave for (10) minutes, then filter the solution and then measure the pH of the three solutions using a PH meter.

Evaluation of the Effectiveness of Extracts

To evaluate the effectiveness of plant extracts on the fungal isolates included in the study, calculate the percentage of inhibition for each extract and use the method of mixing the extract with the agricultural medium (PDA). The culture medium was prepared by sterilizing it in an Autoclave autoclave and leaving it to cool at 40°C. After that, concentrations of 25 and 50 mL of aqueous extracts were added to the PDA culture medium to obtain the required concentrations. After that, it was poured into plates and moved by hand to mix the extract with the medium and left to cool. The dishes were inoculated with the pathogenic fungi under study from a 7-day-old farm. Three replicates were used for each treatment and dishes were inoculated with the pathogenic fungus only without an extract for comparison. The dishes were incubated at a temperature of 26°C and after 10 days of growth the diameter of the fungal colony was measured for all treatments with the control treatment and the percentage of inhibition was calculated [8], using the following equation:

$$\text{Inhibition (\%)} = (C - T)/C \times 100$$

where, C is the average diameter of fungal colony in control treatment and T is the average diameter of the fungal colony grown in the extract.

Evaluation of the Effectiveness of Mixing Extracts

To test the mixing of two types of extracts under study on fungal growth, clove extract and cinnamon were mixed together and clove and ginger were mixed together in proportions of 25+25% and 50+50%, respectively, then the extracts were added to the PDA culture medium to obtain concentrations of 25, 50%. The dishes were inoculated with the pathogenic fungi under study from a 7-day-old farm, three replicates were used for each treatment, the dishes were incubated at a temperature of 26 m and after 10 days of growth the diameter of the fungal colony was measured for all treatments.

Statistical Analysis

The results were statistically analyzed using SPSS. The differences between the means were compared using Duncan's multiple range test at a probability level of 0.05 also the percentage increase between treatments compared to the control treatment was calculated.

RESULTS AND DISCUSSION

Isolated Fungi

The results in Table 1 show the isolation of 42 fungal isolates belonging to 5 genera: *Rhizopus* spp. (5 isolates),

Aspergillus spp. (7 isolates), *Penicillium* spp. (5 isolates), *Fusarium* spp. (11 isolates) and *Alternaria* spp. (14 isolates). *Alternaria* spp. fungus recorded the highest incidence rate of 33.33%. It is worth noting that not all isolated fungi were pathogenic, with the exception of *Fusarium* spp. and *Alternaria* spp., which are both fungi that cause leaf blight. In Iraq, fungi are among the most common causes of rot and wilt diseases in nurseries. These fungi include *Fusarium*, *Pythium*, *Rhizoctonia*, *Alternaria* and *Aspergillus*. These fungi can cause significant economic losses in nurseries due to plant damage [9-11].

Table 2 shows a description of these fungi, as these results agreed with the study of Liu *et al.* [12] in the fungal specifications for fungal spinning and the conidial composition of the fungi under study.

pH of Plant Extracts

Table 3 shows the pH of the aqueous extract of ginger, cloves and cinnamon, which is an important indicator of the extract's content of active compounds. Clove extract was found to be 5.22 more acidic compared to the acidity of cinnamon and ginger, which was 6.10, 6.31, respectively. Their acidity is close to moderate acidity, which is close to 7 and this indicates the presence of compounds of an acidic nature in these plants, such as phenols, terpenoids and volatile oils, which contribute to affecting fungal growth activity.

This is what Chaieb *et al.* [13] pointed out, where clove cabbage contains the compound Eugenol in high concentrations, which is a phenolic compound. Thompson [14] also pointed out in his study that medium and low pH can significantly affect fungal growth as for the cinnamon plant (cinnamon), it contains Cinnamaldehyde and some organic acids that give a relatively low pH value and have inhibitory

Table 1: Percentage of Appearance of Fungi Isolated from Plants Grown in Some Nurseries in Baqubah, the Capital of Diyala Governorate, Iraq

Type of Fungi	No. of isolates	Appearance (%)
Rhizopus spp.	5	11.91
Aspergillus spp.	7	16.66
Penicllium spp.	5	11.91
Fusarium spp.	11	26.19
Alternaria spp.	14	33.33
Total	42	100

Table 2: The Microscopic Diagnosis of Fungi Isolated from Plant Samples Grown in Some Nurseries in Baqubah City

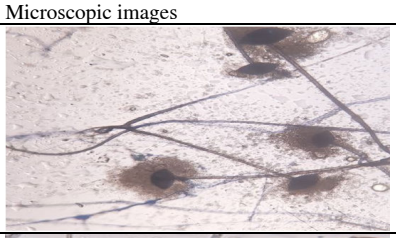
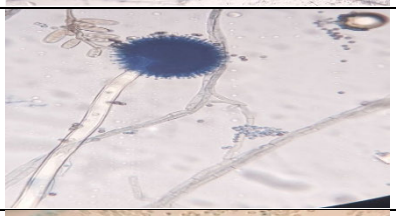
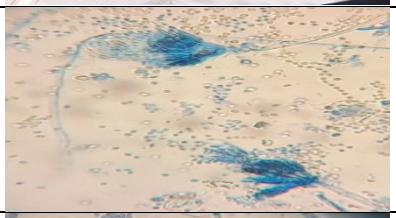
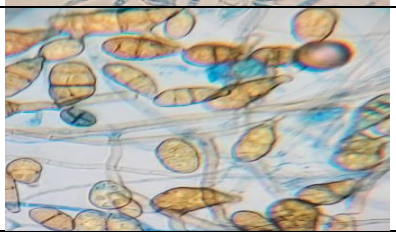
Type of Fungi	Microscopic description	Microscopic images
Rhizopus spp.	The hyphae are septate and unbranched, bearing a large spherical head containing the spores, characterized by root-like sporangiospores.	
Aspergillus spp.	Hyphae are septate, containing a large vesicle bearing small conidia in chains.	
Penicllium spp.	Fungal spores are septa, conidiophores are palmate, bearing conidia and are spherical. Conidia are chain-like.	
Fusarium spp.	Fungal mycelium has septa. The spores are characterized by the formation of two shapes: the large crescent-shaped and the small oval-shaped spores of the type macroconidia and microconidia.	
Alternaria spp.	The fungal mycelium is divided and branched, the conidiophores are short and divided longitudinally and transversely by septa and bear a beak at their ends and the spores are of the multicellular conidia type.	

Table 3: Represents the pH Measurement of Plant Extracts

Extracts	Color	pH
Cloves	dark yellow	5.22
*Cinnamo*n	light yellow	6.10
Ginger	light yellow	6.31

Table 4: The Effect of Aqueous Extracts of Some Plants on *Alternaria* spp. Fungus Growth

Extracts	Concentration (%)	Fungal Culture Diameter(mm)	Inhibition (%)
Cinnamon	25	7.2a	15.29a
	50	5.5b	35.29b
Ginger	25	5.4c	36.47c
	50	3.2d	62.35d
Clove	25	0e	100e
	50	0e	100e
Control	8.5		

Table 5: The Effect of Aqueous Extracts of some Plants on *Fusarium* spp. Fungus Growth

Extracts	Concentration (%)	Fungal Culture Diameter(mm)	Inhibition (%)
Cinnamon	25	5.0a	44.44a
	50	2.3b	74.44b
Ginger	25	8.5c	5.55c
	50	6.3d	30.00d
Clove	25	2.2e	75.55e
	50	1.0f	88.88f

activity for the fungi under study *Alternaria* and *Fusarium* and this is consistent with what Rao and Gan [15] mentioned. As for the ginger plant, it contains gingerol and shogol compounds, which have antioxidant effects and moderate acidity in aqueous solutions and this is consistent with Ali *et al.* [16]. Therefore, the different pH values of plant extracts indicate a variation in chemical composition and this variation affects the effectiveness of these extracts as antifungals or in their use as biological agents.

The Effect of Aqueous Plant Extract on Fungal Growth of *Alternaria* spp.

The results of the plant extracts (cinnamon, ginger and cloves) obtained from their effectiveness on the growth of the fungal colony of *Alternaria* spp. and in comparison, with the control treatment (control) without an extract are shown in Table 4. Where fungal growth was affected by clove extract and this is clear on the diameters of the fungal colony in the two concentrations (25, 50%), with a complete effect, as the diameter of the fungal culture reached (0) mm with a 100% inhibition rate for each concentration and this is consistent with the study of Ahmad *et al.* [17] and with Rana *et al.* [18]. They mentioned widespread activity of cloves against all fungi and clove extract showed moderate to high antifungal efficiency and activity in control.

While cinnamon and ginger extract showed varying inhibition in the average diameters of fungal cultures for the two concentrations 25, 50%, ginger extract recorded the average diameter of the fungal colony (5.4) mm at a concentration of 25%, followed by cinnamon at the same concentration with an average diameter of the fungal colony (7.2) mm and an inhibition rate (36.47, 15.29%) respectively and this is consistent with the study Mohy *et al.* [19], While ginger extract at concentration (50) gave the highest inhibition with a diameter rate of the fungal colony (3.2) mm, followed by the researchers with a diameter rate of (5.5) mm with an inhibition rate of (62.35, 35.29%), respectively, where ginger and cinnamon extracts outperformed it in the inhibition rate in varying proportions. Comparing the results of colony treatments for *Alternaria* fungus treated with extracts with the results of the comparison treatment for a 7-day-old farm without extracts, where the diameter of the fungal colony was (8.5) mm, we find that the plant extracts significantly affected fungal growth and these results explain the effectiveness and inhibitory ability of plant extracts in reducing the spread of fungus and this is clear in Figure 1, as this study agreed with the study of Kisiriko *et al.* [20] with the study of Hyder *et al.* [21], they indicated that ginger extract has the ability to combat pests and fungal pathogens. The reason for this is the ability of active substances found in plants, such as phenolic compounds, terpenes and volatile oils, to inhibit because they have antifungal properties. This study is also consistent with Wijeweera *et al.* [22], who indicated that cinnamon has an inhibitory ability on the growth of a number of pathogenic fungi after being used as an alternative to synthetic fungicides because it contains essential oils such as *Cinnamomum zeylanicum* in the bark, which are concentrated, hydrophobic liquids as they contain volatile chemical compounds from plants and that these essential oils extracted from cinnamon have inhibitory effects on a large number of fungi, as agreed with the study Tabassum and Vidyas [23].

Figure 1 also shows the effect of the aqueous extract of the three plants on the diameter growth of *Alternaria* spp. fungus.

The Effect of Aqueous Plant Extract on Fungal Growth of *Fusarium* spp.

As for the results of Table 5, all extracts showed activity against *Fusarium* fungus, which led to a noticeable decrease in the growth of fungal colonies on Potato Dextrose Agar medium (PDA), where clove extract recorded the highest inhibition superiority, followed by cinnamon extract and then ginger extract in the two concentrations 25, 50% compared to the control treatment (control). Where clove extract gave inhibition at a colony diameter rate of 2.2 mm at a concentration of 25% and 1.0 mm at a concentration of 50% compared to the witness treatment where the colony diameter rate was 9.0 mm and the inhibition rate at the two concentrations was 75.55, 88.88%, respectively, while cinnamon and ginger extract at a concentration of 25%, the colony diameter rate reached (5.0, 8.5) mm and with an inhibition rate of (44.44, 5.55%), respectively. At a concentration of (50)%, cinnamon and ginger recorded a colony diameter rate of (2.3, 6.3) mm with a inhibition rate of (74.44, 30.00%), respectively, compared to the witness treatment The study proved the ability of these extracts to inhibit fungal growth to varying degrees, as this result was consistent with the results of the study of Jeewon *et al.* [24], where he stated in his study that clove extract exerts the greatest anti-fungal growth effectiveness by 100% At all concentrations, the reason is due to the presence of active substances such as glucuronides, glycosides and acidic compounds such as chlorogenic acid dissolved in the

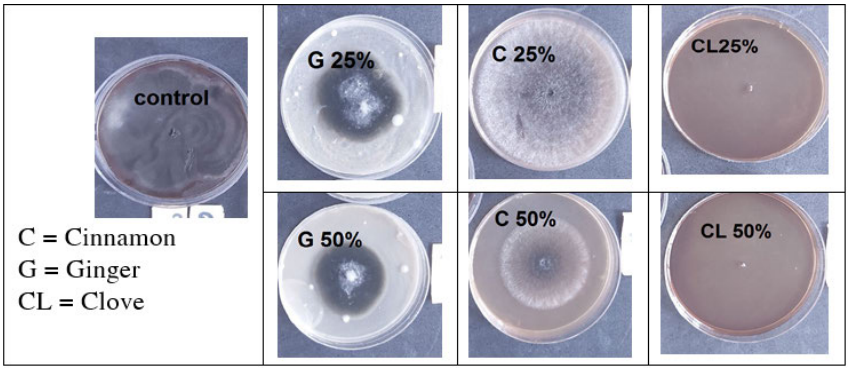


Figure 1: Effect of Plant Extract on Diameters of Colonies of *Alternaria* spp. Fungus

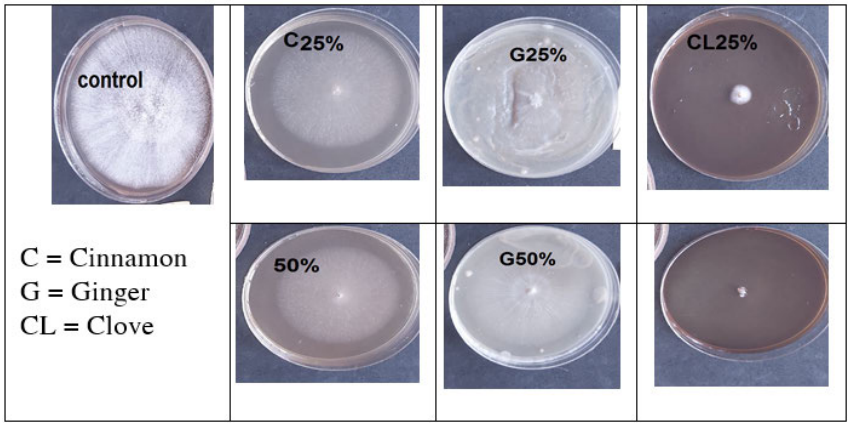


Figure 2: Effect of Plant Extract on Diameters of Colonies of *Fusarium* spp. Fungus

aqueous extract, unlike some studies that focused on essential oils extracted with organic solvents, when they considered that the inhibitory activity is due to the presence of the compound flavonoids and phenols [25,26].

While the aqueous extract has proven its inhibition efficiency due to the presence of other compounds that dissolve in water, this was confirmed by the study of Hassan *et al.* [27], when they mentioned among the other existing compounds myricetin, quercetin, kaempferol and ellagic acid, which have an effect on fungal growth The study also showed the effects of the researchers as an antifungal due to the similarity of its chemical compounds to clove compounds, but at a lower level of inhibition. The difference in the structures of the substances responsible for inhibiting fungal growth may be the reason and this was confirmed by Olamilosoye *et al.* [28], in their study when they indicated that the difference in the proportions of the active substances may be responsible for the decrease in fungal growth activity These results were also consistent with the results of the study of Chrubasik *et al.* [29], where he confirmed that the aqueous ginger extract contains a chemical composition consisting of active ingredients such as carbohydrates, alkaloids, flavonoids, terpenes and phenols, which are compounds that contain antagonistic activity against pathogenic fungi Also, the study of Grzanna *et al.* [30], indicated the effectiveness of ginger against pathogenic fungi, as it indicated that ginger contains approximately 400 chemical compounds, including Gingerols, Zingerone, Shogaols and Sesquiterpenoids, in addition to volatile oils. This is consistent with the current study, as it confirms the effectiveness and ability of the extracts to reduce fungal growth, as shown in Figure 2.

Effect of Interaction between Plant Extracts on Growth of *Alternaria* and *Fusarium* Fungi

Table 6 shows the complete effect of mixing plant extracts on inhibiting the growth of the fungi under study when two types of them are mixed in different treatments. Regardless of the type and concentration of plant extracts used, all treatments showed high effectiveness in inhibiting the fungi *Alternaria* and *Fusarium*, as the inhibition rate was 100% at concentrations (25, 50) % with a colony diameter of (0.00) mm and for all treatments.

It is clear that mixing clove extract with students' extract and mixing clove extract with ginger extract, led to complete inhibition of the growth of the fungi under study. Also, raising the concentration from 25-50% did not show clear differences in effect, which indicates the effectiveness of the mixtures used even at lower concentrations. These results reflect the presence of a synergistic effect between plant extracts when mixed, which led to enhancing their biological effectiveness compared to using only one extract, as indicated by de Almeida *et al.* [31]. In previous studies, clove extract was reported to completely inhibit fungal growth even at the lowest concentration. Compared

Table 6: Effect of Mixing Plant Extracts together on Fungal Growth of *Alternaria* and *Fusarium*

Fungal isolates	Extracts	Concentration%	Fungal Culture Diameter (mm)	Inhibition%
Fusarium spp.	*Cinnamon+ Ginger +Clove*	25	0.00a	100%a
		50	0.00a	100%a
Alternaria spp.	*Cinnamon+ Ginger +Clove*	25	0.00a	100%a
		50	0.00a	100%a
Fusarium spp.	Control	9.0		
Alternaria spp.		8.5		

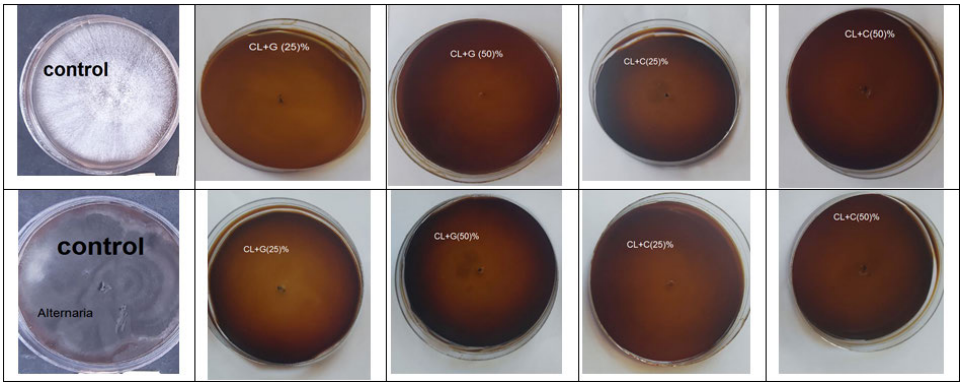


Figure 3: Effect of Mixing Plant Extracts together on Fungal Growth of *Alternaria* and *Fusarium*

to the witness's treatment, the same researcher noted that the active antifungal components associated with cloves appear to be thermally stable, either by preparation in a water bath or by steam sterilization it is an important factor for its applicability in high temperature conditions. A study by Affonso *et al.* [32] showed that clove extract contains eugenol, β-caryophyllene and α-humulene, cinnamon extract contains trans-cinnamaldehyde, which is the predominant compound and ginger extract contains Gingerolo Shogsol as active ingredients with fungicidal activity. However, a large number of studies have shown antifungal activity against Alternaria and Fusarium fungi. It can be mainly attributed to the main active substances found in plants [33-35]. Despite the difference in the physiological and structural composition of the two fungi, the plant extracts mixed together showed equal inhibition efficiency, Figure 3, noting that the effectiveness of the low concentration of 25% was equal to the effectiveness of the concentration of 50% This indicates the possibility of using smaller quantities of different plant extracts mixed together, which is economically and environmentally important, as these extracts have a wide spectrum of fungal effects and can be used as safe natural alternatives instead of traditional chemical pesticides that may have harmful effects on health and the environment [6,17,24].

CONCLUSION

The results indicate a synergistic effect when mixing two types of plant extracts and for all treatments with 100% inhibition of the growth of the fungi under study. This may not be achieved with the same efficiency when using one type of plant extract. However, the low concentration of 25% under study was sufficient to achieve complete inhibition, which allows the field to use lower concentrations of plant extracts in new studies, thus reducing the cost and negative damage to the environment and health when applied to agriculture. The results also proved that the extracts under study have an effective effect on the fungi under study despite the physiological difference between them, as these plant extracts can be used as alternatives to control fungal diseases in plastic homes and prevent the spread of the disease.

Recommendations

The same mixtures of plant extracts can be used on other types of pathogenic fungi to demonstrate the effectiveness of plant extracts. Study these transactions and apply them in the field to know their results more broadly on the plant. Safe plant extracts can be used instead of chemical pesticides because they are environmentally friendly and have lower costs.

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