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Full Length Article

Efficiency of Cinnamon and Thyme Extracts against Bean Root Rot Pathogens with the Presence of some Biological Control Agents

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Abstract

Root rot fungal pathogens cause destructive diseases and threaten broad beans particularly in favorable environmental conditions. Experiments of current study were carried out to examine the efficiency of extracts of both cinnamon and thyme on the growth of pathogenic fungi *Rhizoctonia solani* and *Macrophomina phaseolin*a that cause root rot disease on broad beans in the presence of some biocontrol fungi namely *Trichoderma harzianum* 1, *Trichoderma harzianum* 2 and *Paecilomyces*. Results indicated that *R. solani* and *M. phaseolina* isolated from bean roots reduced broad beans seed germination by over 40% compared to the control treatment and other biocontrol fungi used in the study. Moreover, the severity of infection of broad bean plants by *R. solani* was higher than *M. phaseolina*. A comparison was made between the unsterilized and heat-sterilized powdered extracts for both cinnamon and thyme plants. The outcomes of the study showed that adding unsterilized and heat-sterilized cinnamon powder extract individually reduced the radial growth of both pathogenic and biocontrol fungi in Petri plates. Compared to the other studied fungi, the biocontrol fungus (*Paecilomyces*) was the most affected in terms of radial growth rate by the unsterilized and heat-sterilized cinnamon and thyme powder extracts. The radial growth of *Paecilomyces* in both heat-sterilized cinnamon and thyme powder extract treatments was 1.69 and 1.51 cm, and for the unsterilized extract, it was 1.69 and 1.73 cm, respectively. Results showed no significant differences between adding unsterilized and heat-sterilized cinnamon powder extract. However, the results indicated significant differences between adding unsterilized and heat-sterilized thyme powder extract. Furthermore, the results showed that the radial growth inhibition of fungi increased with increasing concentrations of extracts of both plants, whether unsterilized or heat-sterilized. The study proved that high temperatures did not significantly affect the effectiveness of powdered from both plants studied extracts against the pathogenic fungi.

Keywords: Broad bean; Biocontrol agent; Cinnamon extract; Pathogenic fungi; Thyme

Introduction

Broad bean (*Vicia fabae* L.) is one of the most widely cultivated winter legume crops belonging to the family Fabaceae, which ranks second in importance after the Poaceae family. It is known for its high protein content, estimated around 25–40% **(**Crépon *et al.* 2010), which increases the crop's nutritional value. Additionally, their seeds contain a good percentage of carbohydrates around 51 to 68% in most varieties (USDA 2021). Moreover, broad beans are valued for improving soil properties by nitrogen fixation through root nodules in symbiosis with *Rhizobium* spp. They are often included in crop rotation systems to enhance soil quality (Maluk *et al.* 2022). This important crop is attacked by many agricultural pests, leading to economic losses, such as infestation by insect pests, fungal, viral, and nematode diseases, as well as parasitic flowering plants (Oliveira *et al*. 2014). Root rot disease caused by soil-borne pathogens (*R. solani* and *M. phaseolina*) is one of the most important and common diseases worldwide, including in Iraq, causing economic losses to this crop. They attack plants at all stages of growth, leading to seed rot and seedling death before and after germination. These fungi are among the most dangerous pathogens because they inhabit soil with complex and interrelated relationships with the surrounding environment (Šišić *et al.* 2022). Their danger increases because they grow beyond human perception, and many of them have a wide familial range. They also have the ability to withstand unfavorable environmental conditions and can also have the ability to withstand unfavorable environmental conditions and persist in soil and plant residues for a long time (Agrios 2007).

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Multiple methods have been employed to combat plant diseases; the most important are chemical methods to achieve rapid and decisive results (Butt *et al.* 2020; Thind 2022). However, most chemical fungicides are environmentally polluting, toxic to humans and animals, and negatively affect other soil organisms (Kumar *et al*. 2017). With the increase in the number of fungal strains resistant to chemical pesticides, as well as the decline in the effectiveness of some of these chemicals, agricultural and research institutions have been prompted to seek alternative solutions (Javaid *et al.* 2023). This has led to the use of natural products to control plant pathogens (Ramaiah and Garampalli 2015; Ferdosi *et al*. 2022). The use of plant extracts has gained significant interest among researchers in controlling many fungal plant diseases, as they are naturally occurring substances in plants and possess anti-fungal efficacy against various pathogens (Khan *et al.* 2018; Naqvi *et al.* 2023). These extracts also exhibit desirable properties such as their rapid degradation (Suharti *et al*. 2020). However, it has received significant attention in recent years, as plant extracts and medicinal herbs have been used as sources for producing medicinal drugs or as a source of active ingredients in drug composition (Ferdosi *et al*. 2021; Javaid *et al.* 2022). Many studies have addressed the effect of these extracts on the growth of microorganisms, and thus, they can be used in the treatment of various microbial diseases (Al-Sharmani *et al.* 2019). Iraq is the home to a large number of wild plants, which have significant importance and utmost importance when utilized as antifungal agents and in the production of plant-based pesticides against some causative agents of fungal plant diseases. *Trichoderma* species have been used very commonly as biocontrol agents in addition to other fungal genera such as *Paecilomyces* due to their inhibition effect to wide range of plant pathogens. These biocontrol agents use different mechanisms to eliminate the causes of plant diseases including parasitism, secreting antibiotics, competition for nutrients and space, induced resistance and enhancing the growth of plants (Devi *et al*. 2017). Therefore, the study aimed to isolate and characterize some fungi causing root rot in broad beans and to test the effect of extracts of both cinnamon and thyme on the growth of some pathogenic fungi isolated from broad bean roots with the presence of some biocontrol factors.

Materials and Methods

Collection of plant samples used in the study

Cinnamon and thyme samples were obtained from local markets in Najaf province, and ground using an electric grinder to obtain dry plant powder. The powder was then placed in sterilized paper bags at laboratory temperature until use (Uysal *et al.* 2016). The powdered extracts of cinnamon and thyme were used against pathogenic fungi causing root rot disease in broad beans and biocontrol fungi.

Preparation of potato dextrose agar (PDA)

The medium was prepared by taking 200 g of peeled and chopped potatoes, then boiling them in 500 mL distilled water for 20–30 min in a glass flask. After the boiling period, the mixture was filtered into another glass flask using a piece of gauze cloth to obtain the filtrate. Then, 20 g of dextrose sugar and 17 g of agar were dissolved in another 500 mL of distilled water and the potato filtrate was added. The volume was completed to 1 L and the medium was distributed into glass flasks as needed. The flasks were sealed with cotton plugs and sterilized using an autoclave at a temperature of 121°C and a pressure of 103.4 kPa for 20 min. After sterilization, the flasks were left to cool. Then, 250 mg L^{-1} of the antibiotic Chloramphenicol was added, and the medium was poured into Petri dishes according to the required experiment or stored in the refrigerator until use. This medium was used to grow the studied fungi.

Isolation of fungi used in the study

The fungi were isolated from the roots and crown area of diseased broad bean seedlings. The affected parts were washed with running water to remove soil particles. Then, they were sterilized with a 1% sodium hypochlorite solution prepared from a 6% stock solution for 3 min. Afterward they were rinsed several times with sterile distilled water and placed on sterilized filter paper to remove excess water. The affected parts were then cut into small pieces approximately 0.5–1 cm in length and inoculated onto sterilized Potato Dextrose Agar (PDA) plates. These plates were then incubated in a growth chamber at a temperature of 25 ± 2°C. After fungal growth, they were purified and identified in the Plant Pathology Laboratory of the Department of Plant Protection, Faculty of Agriculture, University of Kufa, based on the characteristics described by Parmeter and Whitney (1970) and Poudel and Vaghefi (2023).

Biological control agents used in the study

Two isolates of the fungus *T. harzianum* and one isolate of fungus *Paecilomyces* were used. These isolates were obtained from the Biotechnology Laboratory, Faculty of Agriculture, University of Kufa, because they have shown high efficacy in previous studies as biological control agents against many plant pathogens and also in promoting plant growth (Ozbay and Newman 2004; Moreno-Gavíra *et al.* 2020).

Propagation of fungal inoculum used in the study

Clean millet seeds were prepared and soaked in water for 6 h. Then, they were placed on filter paper to remove excess water. Seeds were distributed into 250 mL glass bottles at a rate of 100 g per bottle. Bottles were then sealed with cotton plugs and sterilized in an autoclave at a temperature of 121°C and a pressure of 103.4 kPa for one hour. Afterward,

they were taken out and left to cool. The sterilization process was repeated the following day, exactly as in the first time (Dewan 1989). After sterilization, the seeds were taken out from the sterilization device and left to cool. Each bottle was inoculated separately by placing 5 discs with a 0.5 cm diameter for each colony of the studied fungi individually grown on Potato Dextrose Agar (PDA) medium for 7 days individually. Bottles were then incubated at a temperature of 25 ± 2°C for 10 days, shaking them every 3 days to ensure ventilation and distribution of the fungus on all seeds.

Pathogenicity test of studied fungi

The fungal isolates were prepared by using local millet (*Panicum millaceum* L.) seeds, as mentioned earlier. Soil was collected randomly from areas previously cultivated with broad beans at depths of 0 – 30 cm. Then, 5 g of each fungal isolate inoculum, loaded onto millet seeds, were added to 1000 g of soil placed in plastic pots each measuring 25 × 20 cm. The control treatment was carried out using sterilized millet seeds only, following the same procedure. Subsequently, broad bean seeds were sown, with 5 seeds per pot, on the surface of sterilized seeds soaked in a 1% sodium hypochlorite solution (from the original concentration of 6%) for 3 min. The pots were then watered cautiously after random distribution under natural conditions, with attention to maintaining soil moisture through watering as needed (Hassan *et al.* 2013). The germination percentage was calculated after 10 days of planting, and the percentage of infection severity was calculated after 30 days of planting using the following equations:

$$\text{Germination (\%)} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \text{ x } 100$$

The severity of infection was calculated according to the recommended disease index for roots and according to the equation proposed by McKinney (1923). It is as follows:

0 = Healthy roots, 1 = Root contamination, 2 = Contamination of secondary roots and part of the main root, 3 = Contamination of the main root without contamination of the stem base, 4 = Contamination of the main root, wilting and discoloration of the stem base, 5 = Plant death.

Percentage of disease severity = (Number of plants in grade 0 × 0) + (Number of plants in grade 1 × 1) + (Number of plants in grade 2 × 2) + ... + (Number of plants in grade 5 × 5) /Total number of plants examined × 5 × 100

Effect of cinnamon and thyme extracts on the growth of fungi in petri plates

After solidification of the PDA, nutritional medium containing powder of cinnamon and thyme extracts added at concentrations of 0, 5 and 10 g L^{-1}, the petri dishes were inoculated. Each concentration and extract were individually applied as discs with a diameter of 0.5 cm from the edge of colonies aged 7 days for each fungus used in the study, with three replicates for each concentration. A comparison was made with the PDA, nutritional medium alone. Plates were incubated at a temperature of 25 ± 2°C, and the radial growth distance was measured after 7 days of incubation using a transparent ruler.

Effect of adding heat-sterilized extract of cinnamon and thyme on the growth of fungi in Petri plates

The centers of the Petri plates were inoculated after the solidification of the PDA. nutrient medium containing the powders of cinnamon and thyme, added to the nutritional medium after sterilization in an autoclave at a temperature of 121°C and a pressure of 103.4 kPa inch for 20 min, at concentrations of (0, 5, 10) g L^{-1}. Each concentration and extract was separately poured into petri dishes. The centers of the petri dishes were inoculated after solidification of the PDA. nutritional medium containing the powdered extracts of cinnamon and thyme, with each concentration and extract applied individually as 0.5 cm discs from the edge of colonies aged 7 days for each fungus used in the study, with three replicates for each concentration. A comparison was made with the PDA. nutritional medium alone. The dishes were incubated at a temperature of 25 ± 2°C, and the radial growth distance was measured after 7 days of incubation using a transparent ruler.

Statistical analysis

The laboratory experiments were conducted according to the completely randomized design, and mean comparisons were made using the least significant difference (LSD) test at a significance level of 0.01. The field experiment was designed according to the randomized complete block design, and mean comparisons were made using the LSD test at a significance level of 0.05. Each number in the following tables represents the average of three replicates (Mathews and Crossa 2023).

Results

The pathogenicity of isolated fungi

R. solani and *M. phaseolina* isolated from broad bean roots led to a reduction in seed germination percentages of 46.66 and 40%, respectively, significantly different from the control treatment, which reached a germination percentage of 93.33%. Conversely, the fungi *T. harzianum* 2 and *Paecilomyces* increased the germination percentage to 100% each (Fig. 1).

Effect of adding heat-sterilized cinnamon extract on the growth of fungi in petri plates

The outcomes of Table 1 indicated that adding heat-sterilized cinnamon powder extract to the nutritional medium, as previously mentioned, resulted in the inhibition

of radial growth for all studied fungi in Petri dishes. The fungal species *Paecilomyces* was the most affected by cinnamon powder extract, as the extract reduced its radial growth when cultured on the nutritional medium supplemented with cinnamon powder extract after sterilization, with an average effect rate of 1.69 cm. When studying the concentration effect, it was found that increasing the concentration inhibited the radial growth of the studied fungi, with the concentration of 10 g L^{-1} being the most inhibitory, with a radial growth of 0.78 cm Fig. 2. Regarding the interaction between fungi and, the concentration of cinnamon powder extract, adding the powder to the medium had a clear effect on the radial growth of all fungi and for some studied concentrations, resulted in complete inhibition of fungal growth, reaching 0.0 cm for each.

Effect of adding heat-sterilized thyme extract on the growth of fungi in Petri plates

The results of Table 2 indicated that adding thyme powder extract to the nutritional medium after sterilization led to the inhibition of radial growth for all studied fungi in Petri plates. The fungus *Paecilomyces* was the most affected by thyme powder, as the extract reduced its radial growth when cultured on the nutritional medium supplemented with thyme powder after sterilization, with an average effect rate of 1.51 cm. When studying the concentration effect, it was found that increasing the concentration inhibited the radial growth of the studied fungi, with concentrations of 5 and 10 g L^{-1} being the most inhibitory, resulting in 0.0 cm radial growth for all of them. Regarding the interaction between fungi and the concentration of thyme powder, adding the powder to the medium had a clear effect on the radial growth of all fungi and for both concentrations of 5 and 10 g L^{-1}, resulting in complete inhibition of fungal growth, reaching 0.0 cm for each.

Effect of adding non-sterilized cinnamon extract on the growth of fungi in Petri plates

Non-sterilized cinnamon powder extract led to the inhibition of radial growth for all studied fungi in petri dishes. The fungus *Paecilomyces* was the most affected by cinnamon powder extract, as the extract reduced its radial growth, with an average effect rate of 1.96 cm. When studying the concentration effect, it was found that increasing the concentration resulted in greater inhibition of radial growth for the studied fungi, with a concentration of 10 g L^{-1} being the most inhibitory, resulting in a radial growth of 1.85 cm. Regarding interaction between fungi and, the concentration of cold water extract of cinnamon powder, its effect was clear on the radial growth of all fungi and for some studied concentrations, resulted in complete inhibition of growth for some fungi, reaching 0.0 cm for each (Table 3).

Table 1: The effect of adding heat-sterilized cinnamon powder extracts to the nutritional medium on the growth of pathogenic and biocontrol fungi used in the study in Petri plates

Fungi	Radial growth of fungi (cm) Concentration g L^{-1}			Mean fungi
	0	5	10	
R. solani	8.50	2.75	0.00	3.75
M. phaseolina	8.50	0.00	0.00	2.83
T. harzianum 1	8.50	8.33	3.91	6.91
T. harzianum 2	8.50	8.33	0.00	5.61
Paecilomyces	4.54	0.54	0.00	1.69
Mean concentration	7.70	3.99	0.78	
$LSD_{0.05}$	Fungi = 0.40, conc. = 0.31, Interaction = 0.69			

Table 2: The effect of adding heat-sterilized thyme powder extracts to the nutritional medium on the growth of pathogenic and biocontrol fungi used in the study in Petri plates

Fungi	Radial growth of fungi (cm) Concentration g L^{-1}			Mean fungi
	0	**5**	**10**	
R. solani	8.50	0.00	0.00	**2.83**
M. phaseolina	8.50	0.00	0.00	**2.83**
T. harzianum 1	8.50	0.00	0.00	**2.83**
T. harzianum 2	8.50	0.00	0.00	**2.83**
Paecilomyces	4.54	0.00	0.00	**1.51**
Mean concentration	**7.70**	**0.00**	**0.00**	
LSD $_{0.05}$	Fungi = 0.048, conc. = 0.037 , Interaction = 0.048			

Table 3: The effect of adding non-sterilized cinnamon powder extracts to the nutritional medium on the growth of pathogenic and biocontrol fungi used in the study in Petri plates

Fungi	Radial growth of fungi (cm) Concentration g L^{-1}			Mean fungi
	0	**5**	**10**	
R. solani	8.50	1.40	0.00	**3.30**
M. phaseolina	8.50	0.00	0.00	**2.83**
T. harzianum 1	8.50	7.73	5.23	**7.15**
T. harzianum 2	8.50	7.96	4.06	**6.84**
Paecilomyces	4.54	1.36	0.00	**1.96**
Mean concentration	**7.70**	**3.69**	**1.85**	
LSD $_{0.05}$	Fungi = 0.17 , conc. = 0.13 , Interaction = **0.30**			

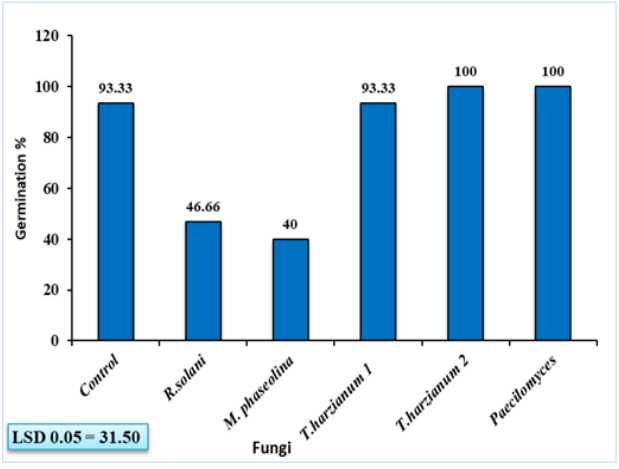

Fig. 1: The pathogenicity of pathogenic fungi to bean roots and some biocontrol fungi in soil placed in plastic pots

Table 4: The effect of adding non-sterilized thyme powder extracts to the nutritional medium on the growth of pathogenic and biocontrol fungi used in the study in petri plates

Fungi	Radial growth of fungi (cm) Concentration g L^{-1}			Mean fungi
	0	**5**	**10**	
R. solani	8.50	8.50	5.73	**7.57**
M. phaseolina	8.50	4.90	3.30	**5.56**
T. harzianum 1	8.50	2.63	0.00	**3.71**
T. harzianum 2	8.50	7.50	1.46	**5.82**
Paecilomyces	4.54	0.66	0.00	**1.73**
Mean concentration	**7.70**	**4.83**	**2.09**	
$LSD_{0.05}$	Fungi = 0.42, conc. = 0.32, Interaction = 0.73			

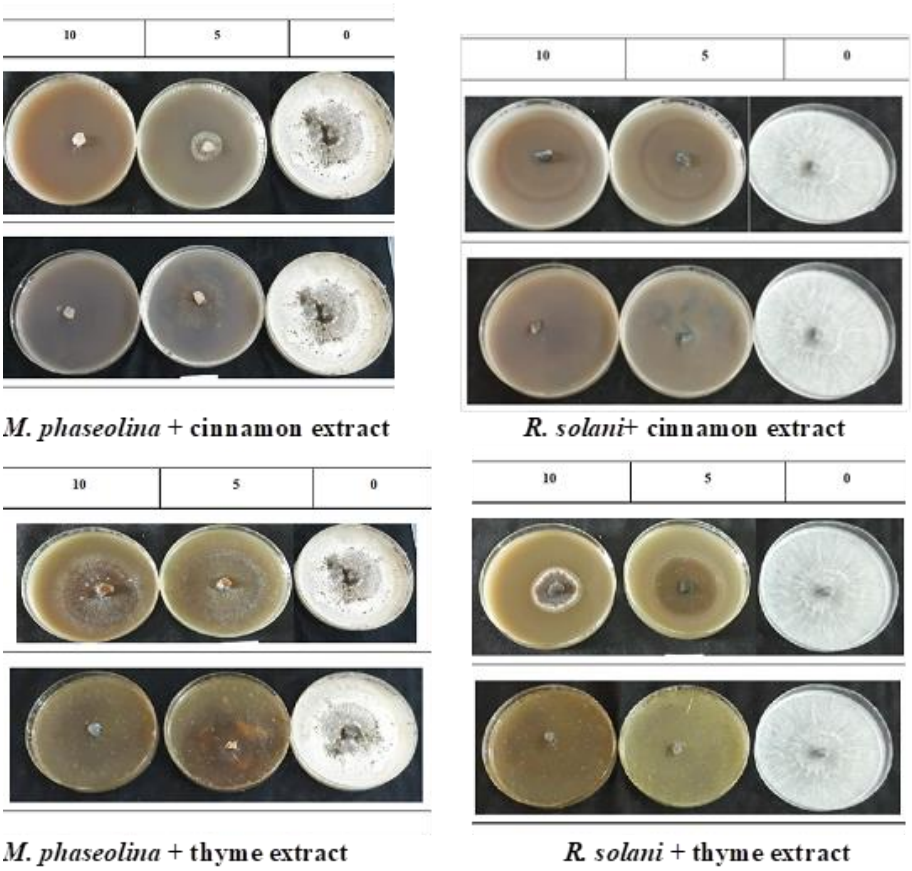

Fig. 2: The effect of different concentrations of cinnamon and thyme extracts on the radial growth of *R. solani* and *M. phaseolina* (the upper section of each image represents before heat-sterilization and the lower section represents after heat-sterilization)

Effect of adding non-sterilized thyme extract on the growth of fungi in Petri plates

Cold water extract of thyme powder led to the inhibition of radial growth for all studied fungi in Petri dishes. The fungus *Paecilomyces* was the most affected by the cold-water extract of thyme powder, as the extract reduced its radial growth with an average effect rate of 1.73 cm. It was found that increasing the concentration resulted in greater inhibition of radial growth for the studied fungi, with a concentration of 10 g L^{-1} being the most inhibitory, resulting in a radial growth of 2.09 cm. Regarding the interaction between fungi and the concentration of cold water extract of thyme powder, its effect was clear on the radial growth of some fungi and, for a concentration of 10 g L^{-1}, resulted in complete inhibition of growth for the fungi *T. harzianum 1* and *Paecilomyces*, reaching 0.0 cm for each (Table 4).

Effect of treatment method and concentration of cinnamon extract on the growth of fungi in Petri plates

There was no significant difference between adding

cinnamon powder to the nutritional medium before and after sterilization. This suggests that cinnamon powder is not affected by heat during the sterilization process, as it effectively inhibited the growth of all fungi, with an average effect rate of 4.22 cm for pre-sterilization treatment and 4.16 cm for post-sterilization treatment. When studying the fungal factor, it became evident that cinnamon powder led to the inhibition of radial growth for all studied fungi in Petri plates. *Paecilomyces* was the most affected by cinnamon powder, as the extract reduced its radial growth with an average effect rate of 1.69 cm. Regarding the concentration effect, increasing the concentration resulted in greater inhibition of radial growth for the studied fungi, with a concentration of 10 g L^{-1} being the most inhibitory, resulting in a radial growth of 1.32 cm. Regarding the interaction between treatment method fungi and, cinnamon powder concentration, adding the powder to the medium had a clear effect on the radial growth of all fungi and for some studied concentrations, resulted in complete inhibition of fungal growth, reaching 0.0 cm for each (Table 5).

Effect of treatment method and concentration of thyme extract on the growth of fungi in Petri plates

The results shown in Table 6 indicated a significant difference between adding thyme powder to the nutrient medium before and after sterilization. This suggests the susceptibility of thyme powder to heat as a result of the sterilization process, where it acted to inhibit the growth of all fungi. The average effect of the treatment was 4.89 cm for the treatment before sterilization and 2.56 cm for the treatment after sterilization. Regarding the fungal factor, it became evident that thyme powder led to a reduction in radial growth for all the fungi studied in Petri dishes. *Paecilomyces* was the most affected fungus by thyme powder, as the extract reduced its radial growth to an average of 1.51 cm. When studying the concentration factor, it was observed that the inhibition of radial growth for the studied fungi increased with higher concentrations. The concentration of 10 g L^{-1} was the most inhibitory for fungal growth, with radial growth reaching 1.06 cm. In terms of interaction between the treatment method, fungi and concentration of cinnamon powder, adding the powder to the medium had a clear effect on the radial growth of all fungi and for some of the studied concentrations, caused complete inhibition of fungal growth, reaching 0.0 cm for each.

Discussion

Results of current study indicated that both pathogens (*R. solani* and *M. phaseolina*) reduced the germination percentage of bean seeds to more than 40%. The pathogenicity test of these pathogens on broad bean showed that *R. solani* was higher in comparison with *M. phaseolina*. These results align with the findings of Amza (2018), which showed that most of the tested fungi causing plant root rot disease significantly reduced seed germination in the planting medium compared to the control treatment. The pathogenicity of the isolates is attributed to their secretion of various toxic secondary metabolites, leading to embryo death and the production of degrading enzymes responsible for seed decay (Ogórek 2016).

R. solani that isolated from bean roots in this study showed high severity on seeds of bean causing them to rot and preventing germination. It also infects seedlings before emergence, leading to a significant reduction in the percentage of germinated seeds by either killing the seeds or weakening the seedlings and delaying their emergence. This results in root rot, lesions, and constriction of the stem bases of seedlings near the soil surface, causing them to fall after emergence above the soil surface and eventually die (Agrios 2005). This confirms previous studies indicating that the fungi *R. solani* and *M. phaseolina* are among the leading pathogens causing damping-off disease in different plant species (Ojo and Olaniran 2015; Rafiq *et al.* 2021). The reason for the decrease in the germination percentage is attributed to the secretion of certain substances by the fungi that either directly degrading the seed coat, thereby aiding in faster germination, or release stimulatory substances that promote germination and growth (Li *et al.* 2019; Jiang *et al.* 2022). Additionally, the study showed that the severity of infection by the pathogenic fungus *R. solani* was higher compared to the infection severity caused by the pathogenic fungus *M. phaseolina*, reaching 4 and 3%, respectively.

The effect of aqueous extracts from some plants on the growth of fungi has been mentioned in several studies. For instance, Al-Askar and Rashad (2010) pointed out that the aqueous clove (*Syzygium aromaticum*) significantly affected the growth rate of the fungus *R. solani*. The inhibition may be attributed to the presence of toxins or antimicrobials as secondary metabolites of invading or parasitic fungi on plant tissues. Crushing those tissues and extracting them (antimicrobial) led to a reduction in radial fungal growth and inhibited their activity (Firáková *et al*. 2007). The effectiveness of these extracts in inhibiting the radial growth of pathogenic fungi may also be attributed to the presence of chemical compounds in these plants that negatively affect the growth of *R. solani* fungus. These compounds are released when added to the growth medium, altering its natural properties and making it less conducive to fungal growth. Additionally, Tegegne and Pretorius (2007) indicated that using a dry powder of *Dolichos kilimandscharicus* and *Maerua subcordata* roots as well as *Phytolacca dodecandra* led to inhibited mycelial growth of three of the six test pathogenics. These results are consistent with those reported by Hakkou and Bouakka (2015), who found that when using extracts of nutral powdered fruits and leaves of rosemary, pomegranate and

Table 5: The effect of treatment method and concentration of cinnamon powder extract on the growth of pathogenic and biocontrol fungi used in the study in Petri plates

Treatment Method with Extract	Fungi	Radial growth of fungi (cm) Concentration g L^{-1}			Mean Treatment Method	Mean fungi
		0	5	10		
Addition Without Sterilization	*R. solani*	8.50	1.40	0.00	**4.22**	**3.30**
	M. phaseolina	8.50	0.00	0.00		**2.83**
	T. harzianum 1	8.50	7.73	5.23		**7.15**
	T. harzianum 2	8.50	7.96	4.06		**6.84**
	Paecilomyces	4.54	1.36	0.00		**1.96**
Addition After Sterilization	*R. solani*	8.50	2.75	0.00	**4.16**	**3.75**
	M. phaseolina	8.50	0.00	0.00		**2.83**
	T. harzianum 1	8.50	8.33	3.91		**6.91**
	T. harzianum 2	8.50	8.33	0.00		**5.61**
	Paecilomyces	4.54	0.54	0.00		**1.69**
Mean concentration		**7.70**	**3.84**	**1.32**		
$LSD_{0.05}$	Treatment Method = 0.14, fungi = 0.31, Conc. = 0.14, Interaction = 0.54					

Table 6: The effect of treatment method and concentration of Thyme powder extract on the growth of pathogenic and biocontrol fungi used in the study in Petri plates

Treatment Method with Extract	Fungi	Radial growth of fungi (cm) Concentration g L^{-1}			Mean Treatment Method	Mean fungi
		0	5	10		
Addition Without Sterilization	*R. solani*	8.50	8.50	5.73	**4.89**	**7.57**
	M. phaseolina	8.50	4.90	3.30		**5.56**
	T. harzianum 1	8.50	2.63	0.00		**3.71**
	T. harzianum 2	8.50	7.50	1.66		**5.88**
	Paecilomyces	4.54	0.66	0.00		**1.73**
Addition After Sterilization	*R. solani*	8.50	0.00	0.00	**2.56**	**2.83**
	M. phaseolina	8.50	0.00	0.00		**2.83**
	T. harzianum 1	8.50	0.00	0.00		**2.83**
	T. harzianum 2	8.50	0.00	0.00		**2.83**
	Paecilomyces	4.54	0.00	0.00		**1.51**
Mean concentration		**7.70**	**2.41**	**1.06**	5.73	
$LSD_{0.05}$	Treatment Method = 0.13, fungi = 0.29, Conc. = 0.16, Interaction = 0.50					

oleander as an inhibitor for the fungus *Fusarium oxysporum* f. sp. *albidinis*, the causal agent of vascular wilt disease of date palm, the three extracts inhibited the mycelial growth of fungus. They also agree with the findings of Lengai *et al*. (2020), indicating that some plants contain chemical compounds capable of inhibiting the growth of various microorganisms, different from the pesticides used to control these organisms. These compounds work by reducing the total protein and carbohydrate content and by poisoning the fungi by reducing the effectiveness of the Catalase enzyme in fungal cells, subsequently decreasing the growth rate (Valenzuela-Cota *et al.* 2019).

The radial growth of both biocontrol fungi used in this study was slightly affected by the unsterilized and heat-sterilized cinnamon and thyme powder extracts. However, *Paecilomyces* was the most affected**.** This slight effect of studied extracts on the bio-control fungi may occur due to less synergistic antagonistic effect (Rosil *et al.* 2022).

Conclusion

Cinnamon and thyme extracts can be used against pathogenic fungi that cause root rot disease in broad beans. The results highlighted the significant impact of both cinnamon and thyme extracts, whether sterilized or unsterilized, on reducing the radial growth of all tested fungi in Petri plates. Notably, the biocontrol fungus *Paecilomyces* was particularly affected by both types of extracts. While there were no significant differences observed between adding sterilized and unsterilized cinnamon powder extract, the effectiveness of sterilized thyme powder extract was significantly lower compared to the unsterilized extract. Additionally, the experiment demonstrated that increasing concentrations of both plant extracts led to increased inhibition of fungal growth. Importantly, the study showed that high temperatures did not compromise the effectiveness of the powdered extracts against the pathogenic fungus. Overall, these findings suggest the potential of cinnamon and thyme extracts as effective agents against root rot disease in broad beans, with implications for agricultural practices aimed at disease management and crop protection.

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Author Contributions

UAA Alshimaysawe, HAA and AEM performed experiments, collected data and FHAl-haidary analysed the data. All authors wrote the manuscript and approved the final version equally.

Conflicts of Interest

All authors declare no conflicts of interest

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable

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