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RESEARCH ARTICLE

Antifungal activity of cinnamon and mint extracts against white and basal rot of garlic

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Abstract

Sclerotium cepivorum and Fusarium oxysporum f.Sp cepae are the causal of white rot and basal rot diseases and are the most damaging soil-borne pathogens that threaten garlic (*Allium sativum*) and other Allium species cultivation in Egypt. This study was carried out to test in vitro and *in vivo* the efficacy of the natural extracts as it is considered one of the better alternative methods for disease control using Baladi and Sides 40 cultivars. In vitro trails were organized using potato dextrose agar with adding Cinnamon (*Cinnamomum zeylanicum*) bark extract and Mint (Mentha) leaf extract at concentrations of (10%, 25% and 50%). The two different extracts were used to examine their effect on mycelial growth with reference control petri plate. It was observed that Cinnamon extract was the best plant extract treatment giving inhibition in mycelial growth. On the contrary, mint extract gave low suppression of the mycelia growth. It could be concluded that 50% concentration of cinnamon extract was used *in vivo* experiment as the most effective plant extract and concentration in vitro experiment.

Keywords: *Allium sativum;* Cinnamon extract; *Fusarium oxysporum fsp Cepae;* Mint extract; *Sclerotium cepivorum.*

1. Introduction

Low Garlic (*Allium sativum* L.), an underground bulb crop belonging to the genus Allium of Alliaceae family, is often consumed as a vegetable and used as a seasoning/condiment (Qiu *et al.*, 2022). it is one of the most significant bulb vegetable crops, which is second only to onion (Allium cepa) in importance (Hamma, Ibrahim, Mohammed, and Development, 2013). It is widely applied as a spice or for medicinal uses. It has typically been grown in Egypt for both domestic use and export. Postharvest handling, processing methods, and storage conditions all affect the nutritional value and health properties of garlic-based products (Qiu *et al.*, 2020) Several fungal diseases affect garlic, the most significant of which is white rot *of Sclerotium ceprivorum***,** Fusarium basal rot of *Fusarium oxysporum f.sp. cepae*, pink root rot of *Pyrenocheta terrestris*, rust of *Puccinia allii* and leaf blight of *Stemphylium spp*. (Patón, Marrero, and Llamas, 2017) . A fungus found in the soil causes white rot of garlic and onions. An inoculum density with few soil sclerotia in a field soil litter can result in considerable crop losses (Davis, Hao, Romberg, Nunez, and Smith, 2007). The white rot pathogen can remain dormant as a small, spherical, and black sclerotia on plant debris in the soil for more than 20 years in the absence of Allium crops (Entwistle and management, 1990). In Egypt, *Sclerotium cepivorum*, which caused white rot of onions and

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garlic, was widely prevalent (Embaby, 2003). Among these, basal rot, which is caused by *Fusarium oxysporum f. sp. cepae*, was a widespread and economically significant disease that affects onion, garlic, and several other Allium species, including chive and shallot. It also results in significant yield losses throughout the world (Behrani *et al.*, 2015). The most popular strategies for preventing and treating soil-borne diseases include crop rotation, solarization, fumigation, and chemical fungicides. Even if the use of fungicides provided adequate control over plant diseases, they could accumulate dangerous poisonous chemicals that endanger human life and the environment. Additionally, it has been discovered that pathogens can become resistant to a number of fungicides (Deising, Reimann, and Pascholati, 2008). Plant extracts have demonstrated complementing effectiveness in the management of soil-borne diseases (Javaid, Rauf and Biology, 2015). Another alternative is the use of plant metabolites and plant-based pesticides, which are known to have less of an adverse effect on the environment and pose fewer risks to consumers than synthetic pesticides do (Ramaiah, Garampalli,and Research, 2015).The purpose of this study was to assess the antifungal effectiveness of cinnamon and mint plant extracts against the fungi *Sclerotium cepivorum* and *Fusarium oxysporum f.sp. cepae,* which cause white rot and basal rot, respectively.

2. Material and methods

2.1. Isolation and Identification

Plants which showing up white rot and basal rot symptoms were gathered from different locations in Luxor and Qena Governorates. White rot which cause by *Sclerotium cepivorum* has been isolated as a Sclerotia particles where on the surface of infected garlic bulb and by picking off mycelia growth from diseased garlic as reported by (Clarkson, Payne, Mead, & Whipps, 2002). These Sclerotia dipped in 70% ethyl alcohol for about 2 minutes, washed three times in sterilized distilled water and dried well by using sterilized filter papers then handed over to petri dishes (9 cm in diameter) containing about 15 ml of sterilized Potato Dextrose Agar (PDA) as media, supplemented with streptomycin antibiotic 400 mg/L to prevent any bacterial contamination after that incubated at 20 ºC. Other Diseased plants which show basal rot *(Fusarium oxysporum*) were washed thoroughly under running tap water to remove any adhesive soil particles. Then the diseased basal parts were cut into small pieces of about 5 mm long by using a sterilized scalpel and surface sterilized with dipping in 70% ethyl alcohol for 2 minutes then washed three times carefully in sterilized distilled water. Pieces were dried by sterilized filter paper, then transferred individually into Petri dishes (9 cm in diameter) containing about 15 ml of sterilized (PDA) media, supplemented with streptomycin antibiotic 400 mg/L to avoid any contamination with bacteria. After that Petri dishes incubated at 27°C. All dishes were daily examining to keep an eye on the hyphal growth. Then, the fungal colonies purified using single spore and hyphal tip technique in agreement with (Brown, 1924). The obtained isolates of *S. cepivorum* and *F. oxysporum* were maintained on PDA slants and kept in refrigerator at 5°C for further studies.

2.2. Pathogenicity test

The ability of thirteen isolates of *F. oxysporum* which cause basal rot disease and six isolates of *S. cebivorum* which cause white rot disease on Garlic plants were tested using Baladi and Sides 40 cultivars during winter season under field conditions to determine the pathogenicity potential of the tested fungal isolates. This study was carried out using sterilized plastic pots (25 cm in diameter) by immersing in formaldehyde solution for 15 minutes, then left to dry, to get rid of the deleterious effect of the formaldehyde. Sterilization of the soil executed by wetting the soil by formaldehyde solution and covered with polyethylene layer for 7 days to retain the evaporation caused by formaldehyde to ensure

High sterilization efficiency. Soil did not treat until all residue traces of formaldehyde disappeared (approximately after 4 weeks). Sterilization of the pots and the soil were done by using 5% formaldehyde solution. Inoculum was prepared in 250 ml flasks by (200 g grain sorghum) for each flask. Each one of the flasks inoculated by 5 discs (10 mm made by cork-borer) of 7 days old culture of the tested fungi and incubated at 27 ºC for two weeks, for the *S. cepivorum* isolates incubated at 20 ºC. The sterilized pots were filled with 3 kg sterilized soil. After that, the inoculum was mixed with the soil at the rate of 3% (w/w) of the soil, and then irrigated three times a week before planting to ensure distribution and growth of each isolate. Three garlic cloves of the two cultivars were planted into each infested pot, three replicates were used for each treatment. Results were reported after 90 days of planting for white rot and after 120 days for basal rot. The diseased plants of each replicate were uprooted from the pots at the end of experiment period, and washed thoroughly to get rid of the stucked soil particles.

2.3. Disease index of the plant death

Disease index of foliar yellowing was determined by rating each leaf on the plant for severity of wilt symptoms (yellowing) according to 0-5 scale and computing the average grade for plant as a whole (EL Zawahry, 1984), the following numerical grades were used:

1= healthy plants

2= slightly severe (leaves yellowing, root system reduced)

3= moderate severe (yellowing, die back of leaves and badly decayed root)

4= severe (completely yellowing plant, leaves die back, semi soft rot of cloves and roots)

5= highly severe (completely dead plants, extensive decayed roots and bulbs).

The basal rot rating scale was as follows:

1 = without symptoms

2 = up to 10% rotted roots

3 = 10-30% rotted roots with up to 10% rotted basal plates,

4 = completely rotted roots and 10-30 % rotted basal plates

5 = completely rotted roots and more than 30% rotted basal plate.

For calculating the percentage of disease severity index the following

formula was used (Fakhouri, Buchenauer, and Protection, 2003).

$$DS\ (\%) = \Sigma\ [\ (1A+2B+3C+4D+5E)\ /5T] \times 100$$

Where, A, B, C, D and E are the number of plants corresponding to the numerical grade, 1, 2,3,4 and 5 respectively and 5T is the total number of plants (T) multiplied by the maximum disease grade 5, where T=A+B+C+D +E. To detect the different degrees of disease, plants were classified into five categories according to (Rengwalska and Simon, 1986; Shatla *et al.*, 1980).

2.4. Identification of the pathogenic fungi

The fungal isolates were identified by using the morphological features of mycelia and spores as described by (Barnett and Hunter, 1972; Nelson, Toussoun, and Marasas, 1983). and confirmed by Animal health research institute, Dokki, Giza, Egypt

2.5. In vitro experiment

This study was aimed to evaluate the effectiveness of the extracts of Cinnamon (*Cinnamomum zeylanicum*), against the mycelial growth of *Sclerotium cepivorum and Fusarium oxysporum*

2.6. Plant material

The extracts from Cinnamon and mint were tested against the fungal pathogens. The extracts were prepared from fine powder of cinnamon and mint. The powder was soaked in distilled water at the rate of 1: 2 (w/v) then, the mixture was heated at 100°C for 30 min, and filtered through cheese. The extracts were centrifuged at 12000 rpm for 30 min at 25±3°C and sterilized by filtering through a 0.22 µm membrane filter to avoid any

bacterial or fungal contamination. The extracts were considered as 100% concentration. Then was mixed with PDA at 48ºC to obtain (10, 25 and 50 %, with 0.5% of Tween-80 (v/v)) to enhance oil solubility. The amended media were poured into 9 cm Petri dishes (15 ml per plate). Three replicates for each concentrate were used. Control treatment (0%) was done by mixing PDA with only tween-80 without any other additives. All plates were left for a while to be solidified before inoculation with 5mm disks of pathogen, taken from 7day old culture in the center of each plate. then incubated at (27°C) for *F. oxysporum* and (20°C) for *S. cepivorum*. The Average of radial growth was recorded after 7 days compared with the untreated control percentage when mycelial growth covered the surface of all cultures in the control treatment. Inhibition of growth was calculated in compare of the control, according to the equation proposed by (Pinto, Maffia, Berger, Mizubuti, and Casali, 1998).

2.7. In vivo experiment

These experiments were carried out at the experimental farm of faculty of agriculture, south valley university to study the effect of physical control to protect garlic plants from the infection of *F. oxysporum* and *Sclerotium cebivorum* on (Balady and Sides 40) under field conditions. The efficacy of the promising treatment obtained in vitro trail for controlling root rot diseases of garlic were applied. The field experiment consisted of 3 plots, 12 rows, 4 rows for each fungus (Each row is for a certain cultivar. and 2 rows for the control). The area of the plot ($3m^2$ (3Lx1W) each row sown with 10 garlic cloves as replicates. The experiment was set in completely randomized block design, the treatments were then applied Planting of garlic cultivar was carried out by late November–early December, depending on the experiments. Mineral fertilization, hand weeding, and irrigation (with sprinklers) were conducted according to standard practices. in field and application of bio-agents were carried out at planting date by adding bio-agents in previously infested soil. The two isolate of *F. oxysporum* (F5 and F9) and the isolate of *S. cepivorum* (S3) were grown on autoclaved 250ml flasks with (200g grain sorghum) for each flask. Each one of them inoculated by 5 discs 10 mm from 7 days old culture of (F5, F9 and S3). Then incubated at (20°C) for (S3) and (27°C) for (F5 and F9). After that added to the soil in the rate of 50g/plant. Plant extract solutions were prepared in sterile distilled water to a final concentration of 3 ml L-1, and applied by soaking the garlic cloves before sowing and after adding the pathogens and one more time after 30 days of planting.

3. Results and discussion

3.1. Pathogenicity test

Nineteen fungal isolates were tested to study their pathogenic capability on garlic plants (Balady and Sides 40 cvs) during season. Data showed in Table (1) that all tested fungal isolates of *F. oxysporum* and *S. cepivorum* were able to infect garlic plants (Balady and Sides 40) causing typical symptoms of basal rot and white rot disease, but these isolates were significantly different in their strength to cause these symptoms in compare of the control. Data demonstrated that, *S. cepivorum* (isolate no.3) caused the highest percentage of disease severity in the both cultivars (Balady and Sides 40 cvs) reached to 79% and 83% respectively. Followed by isolates no. 2, 5 and 6 which reached to 77%, 73% and 65% for (Balady cv), and 78%, 77% and 65% in (sides 40 cv) respectively. While isolates No. 4 and 1 in (Balady cv) gave moderate disease severity by 55% and 51% and for (sides 40 cv) gave 58% and 53% correspondingly. on the other hand, all the tested isolates of *Fusarium oxysporum f.Sp cepae* caused basal rot disease in compare of the control.

In this case, isolates no. 5 and 9 gave the highest disease severity in both of the cultivars, 71% and 77% for (Balady cv) and 72% and 79% for (Sides 40) respectively. Isolates no. 3, 6 and 7 gave 60%, 58% and 57% in (Balady cv) and 62%, 60% and 58% relatively. However, isolates No. 10, 4, 1, 2,

12, 13, 11 and 8 gave variant disease severity 55%, 47%, 52%, 50%, 46%, 46%, 43% and 43% for (Balady cv) and 57%, 50%, 46%, 48%, 45%, 47%, 46%, and 40 respectively for (Sides 40 cv).

Table 1. Disease severity of garlic white rot and basal rot diseases caused by 19 fungal isolates during 2019 growing season.

Isolates		Cultivars	
		Baladi	Sides 40
Fusarium oxysporum	F1	52	46
	F2	50	48
	F3	60	62
	F4	47	50
	F5	71	72
	F6	58	60
	F7	57	68
	F8	43	40
	F9	77	79
	F10	55	57
	F11	43	46
	F12	46	45
	F13	46	47
Sclerotium cepivorum	S1	51	53
	S2	77	78
	S3	79	83
	S4	55	58
	S5	73	77
	S6	65	65

According to the obtained data, *Sclerotium* isolate No. (3) and *Fusarium* isolates No. (5 and 9) were selected under laboratory and field conditions. Such results are in agreement with those obtained by (Dilbo, Alemu, Lencho, Hunduma, and Microbiology, 2015; Rout, Tripathy, Nanda, Nayak, and Joshi, 2016) who found *that F. oxysporum f. sp. cepae* and *S. cepivorum* caused basal rot and white rot diseases of garlic under greenhouse and field conditions.

3.2. In vitro experiment

The result in table (2) illustrate that Cinnamon extract was the most effective on radial growth suppression of *F. oxysporum f. Sp cepea* and *S. cepivorum* giving at concentration 10% (F5=60%, F9=57% and S3=65%), in 25% gave (F5=73%, F9=69% and S3=88%) and in 50% concentration gave (F5=80%, F9=75% and S3=95%) reduction in mycelium growth and sclerotia germination in compare of the control. This result is agreed with the results of many authors who reported that, the antifungal activity of cinnamon extract against plant pathogenic fungi, they reported that cinnamon extract inhibited the radial growth of *B. cinerea*. .This antifungal activity of cinnamon extract due to be presence of some active compounds such as (cinnamaldehyde, eugenol, cinnamic acid, weathering) (Wilson, Solar, El Ghaouth, and Wisniewski, 1997). On the contrary mint extract gave moderate reduction of the radial growth of the two under study fungi at all of the tested concentrates respectively. At concentration 10% (F5=36%, F9=27.5% and S3=33%), in 25% gave (F5=44.5%, F9=50% and S3=46%) and in 50% gave (F5=56.7%, F9=73% and S3=58%). This result contradicts (Jadon, Shah, and Protection, 2012) who reported the power of the mint extract to suppress the fungal growth.

Table 2. In vitro experiment of different concentrations of Cinnamon and mint extracts on (F5, F9 and S3) isolates.

Plant extract	F5			F9			S3		
concentration	10%	25%	50%	10%	25%	50%	10%	25%	50%
Cinnamon	60	73	80	57	69	75	65	88	95
Mint	36	44	56	27	50	73	33	46	58

3.3. In vivo experiment

50% concentration of cinnamon extract was used *in vivo* experiment. The choice was based on the most effective plant extract and concentration in vitro experiment. The ability of the cinnamon extract to protect garlic plants against white and basal rot diseases was studied using (balady and sids40) under field conditions during 2020 and 2021 growing seasons. Data in Table (3) showed that the treated soil with cinnamon extract significantly reduced the disease severity of garlic disease compared with the control. The results of disease severity reduction reached to (F5=14%, F9=17% and S3=15%) for (Baladi cv) While, (Sides 40 cv) reached to (F5=15%, F9=17% and S3=16%). These result are in agreement with those obtained by (El-Sheshtawi, El-Gazzar, Saad, and Pathology, 2009).

Table 3. In vivo experiment with 50% concentrations of cinnamon extracts on (F5, F9, and S3) isolates and on Baladi and Sides 40 cultivars.

Cultivars	F5	F9	S3
Baladi	14	17	15
Sides 40	15	17	16

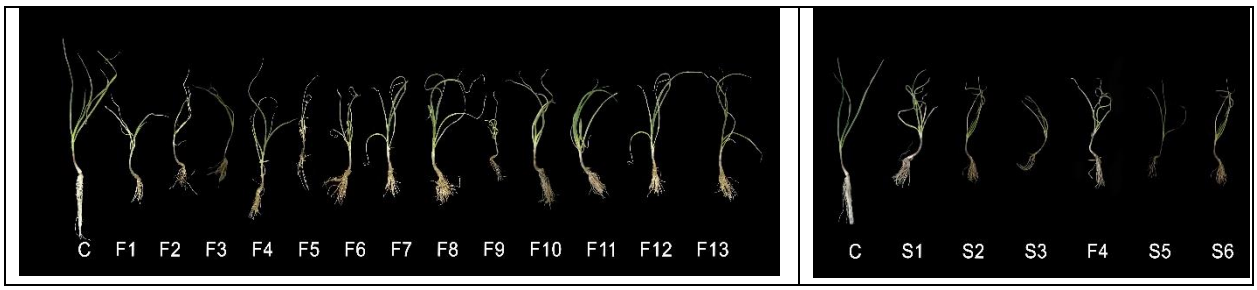

Figure 1. Disease severity of garlic basal rot and white rot diseases on Baladi cultivar.

Figure 2. Disease severity of garlic basal rot and white rot diseases on Sides 40 cultivar.

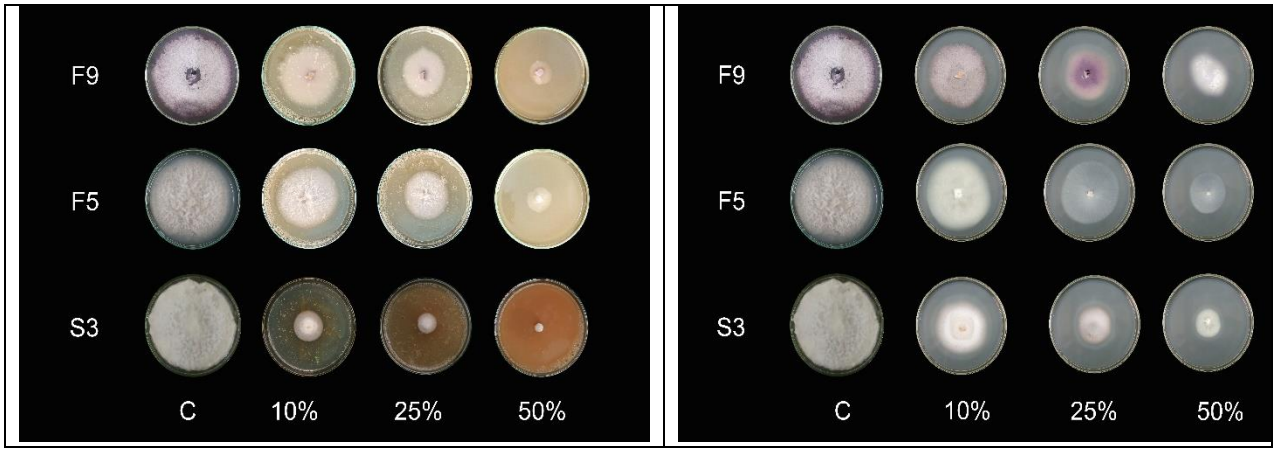

Figure 3. in vitro experiment with different concentrations of cinnamon and mint extracts on (F5, F9, and S3) isolates.

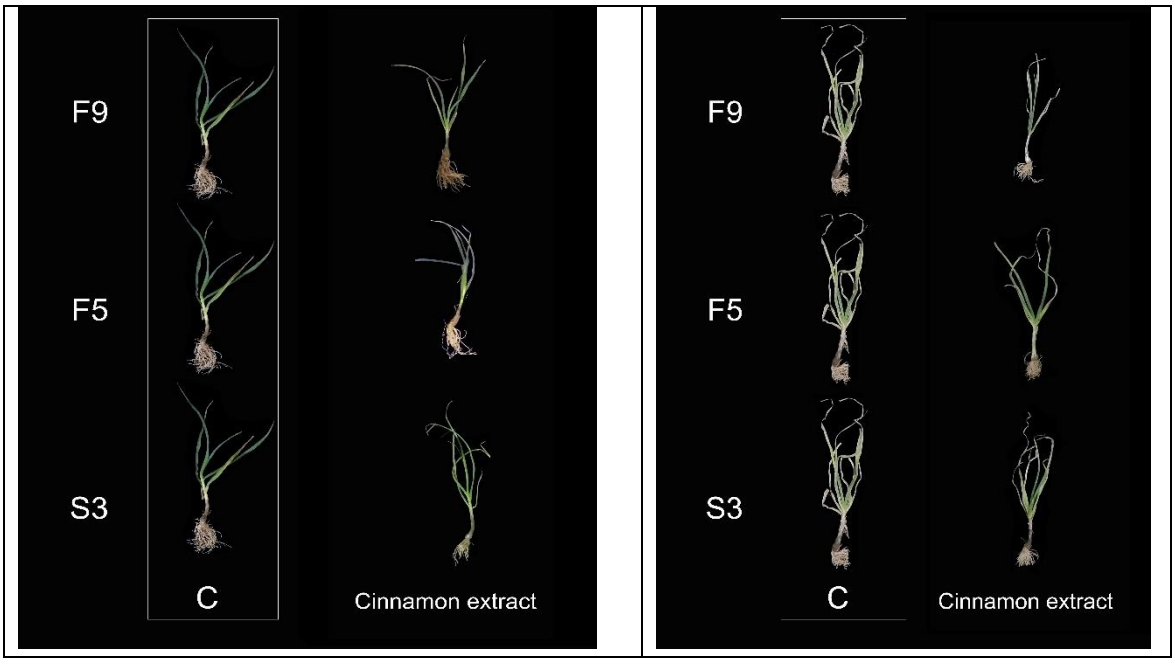

Figure 4. In vivo experiment with 50% concentrations of cinnamon extracts on (F5, F9, and S3) isolates and on Baladi and Sides 40 cultivars

4. Conclusion

It could be concluded that 50% concentration of cinnamon extract was used in vivo experiment as the most effective plant extract and concentration in vitro experiment.

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All authors are contributed in this research.

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Institutional Review Board Statement

All Institutional Review Board Statements are confirmed and approved.

Data Availability Statement

Data presented in this study are available on fair request from the respective author.

Ethics Approval and Consent to Participate

Not applicable

Consent for Publication

Not applicable.

Conflicts of Interest

The authors disclosed no conflict of interest starting from the conduct of the study, data analysis, and writing until the publication of this research work.

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