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Antifungal Activity of Medicinal Plant Extracts Against Postharvest Fungi of Fruits and Vegetables with in vivo Protection of Tomato Fruits

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ABSTRACT

This study aims to examine the effect of certain medicinal plant extracts on some fungi isolated from post-harvest fruits and vegetables, and the likely use of these extracts to extend the time that fruits and vegetables are protected from fungus contamination during preservation. The plant extracts, which were used in the current study, included clove (*Syzygium aromaticum*), cinnamon (*Cinnamomum zeylanicum*), anise (*Pimpinella anisum*), wild thyme (*Thymus serpyllum*), bay leaves (*Laurus nobilis*), and cumin (*Cuminum cyminum*). The fungi isolated from fruits and vegetables included *Aspergillus niger, Rhizopus stolonifer, Fusarium oxysporum, and Penicillium expansum* with the goal of achieving sustained development in plant pathogen control. The greatest decrease in growth was recorded at 100% concentration of all extracts for all fungi. The results also showed that clove and cinnamon extracts were superior to the other extracts used, exhibiting the highest effect in reducing fungal diameters. The inhibition ratio for clove extract at 100% concentration were 64.14%, 54.07%, 51.41%, 58.71% against *A. niger*, *R. stolonifer*, *P. expansum*, and *F. oxysporum*, respectively. For cinnamon extract at 100% concentration, the corresponding inhibition ratios were 76.13%, 63.71%, 72.20%, 70.72% against the same fungi. Clove and cinnamon extracts also showed superior effects in reducing fresh and dry biomass. For clove extract, the inhibition rates in fresh weight were 36.78%, 26.37%, 36.55%, 12.50%, while those in dry weight were 37.25%, 32.65%, 26.92%, and 27.65% for *A. niger*, *R. stolonifer*, *P. expansum*, and *F. oxysporum*, respectively. For cinnamon extract, the inhibition rates in fresh weight were 49.42%, 42.85%, 47.31%, and 22.72%, and the inhibition rates in dry weight were 56.86%, 36.73%, 55.76%, and 25.53% against the same fungi, respectively. And the highest efficiency in preserving tomatoes in storage for the longest possible period, this observed through decreasing the inhibition severity after treatment with plant extract which gave; 23–40% for *A. niger*, 25–40% for *F. oxysporum*, 23–50% for *R. stolonifer*, and 20–50% for *P. expansum* comparing with inhibition severity before treatment with plant extracts which gave, from 40–65% for *A. niger*, 40–65% for *F. oxysporum*, 50–70% for *R. stolonifer*, and 40–70% for *P. expansum*, while the control treatment recorded the highest infection rates of 70–80% for all tested fungi.

1. INTRODUCTION

Post-harvest diseases pose a global challenge that directly affects food security, as pathogenic fungi cause losses ranging from 20–50% of fruit and vegetable crops in developing countries. Tomato fruits (*Solanum lycopersicum*) are among the most susceptible plant hosts to rapid spoilage due to their high moisture and nutrient content, making them an ideal environment for the growth of opportunistic fungi, such as *Alternaria alternata*, *Botrytis cinerea*, and *Rhizopus stolonifer*, which are widespread in local markets and secrete *mycotoxins* that pose a risk to public health [1, 2].

Protection strategies have relied on synthetic fungicides, while the indiscriminate use of these compounds has resulted in serious repercussions, including the emergence of resistant fungal strains, the accumulation of toxic residues in fruits, and the disruption of the ecological balance, prompting the scientific community to search for safe and environmentally friendly alternatives [3, 4].

Consequently, plant extracts rich in secondary metabolites have emerged as a promising strategy for biological control due to their high inhibitory efficacy and rapid biodegradability. To accurately assess the efficiency of these extracts, it is insufficient to depend entirely on measuring the apparent diameter of the colony (Radial growth) in Petri dishes. Rather, it is essential to examine their effect on the biomass of the fungus represented by fresh weight and dry weight. The reduction in dry weight is a precise biological marker that reflects the interaction of plant compounds with the metabolic processes and synthesis of the fungus's cell wall [5, 6].

The research problem lies in the rapid deterioration of fruits and vegetables in local markets due to fungal infections,

leading to significant economic losses and a decline in market value. Despite the effectiveness of chemical pesticides, their continuous use has resulted in the accumulation of toxins, leading to the emergence of resistant fungal strains, posing a direct threat to consumer health and the environment.

This study aims to isolate fungi contaminating vegetables and fruits in local markets, evaluate the effectiveness of certain plant extracts in many concentrations (25, 50, 75, 100%) in inhibiting fungi growth diameter, affect the plant extracts with concentration 100% on biomass (fresh and dry weight), and examine their practical (in vivo) application to protect tomato fruits from artificial inoculation. In so doing, it paves the way for the development of plant-based fungicides that are safe for consumption. The unique contribution of this study lies in its comprehensive approach to evaluating the antifungal activity of plant extracts. By simultaneously measuring radial growth, biomass: conducted biomass measurement as a complementary indicator to radial growth, which remains the most commonly used parameter in antifungal assays and viable protection, we can gain a more complete understanding of how these extracts inhibit fungal growth and protect against infection. This multi-faceted approach allows us to:

- Assess the direct impact on fungal growth (radial growth and biomass).
- Evaluate the protective effect against infection (viable protection).

This comprehensive evaluation will provide a more robust understanding of the extract's potential as a postharvest disease management solution.

2. MATERIALS AND METHODS

2.1 Plant sample collection and extract preparation

The plant parts, clove flowers, cinnamon bark, bay leaves, cumin seeds, anise seeds, and wild thyme flowers were collected from local markets (Table 1) and milled into a fine powder to show the important effect on pathogenic fungi. Extraction was performed using the cold maceration method as described by research [7], 5 g of the plant powder were infused in 50 mL of sterile distilled water for 48 hours using a shaker in the lab. temperature (25 ℃) and the extraction was repeated twice. The mixture was filtered using Whatman No. 1 filter paper to obtain the crude extract (100%) from which all other concentrations were prepared: 0, 25, 50, 75, 100% [8]. The extract should be stored in the refrigerator at 4 ℃.

Table 1. Plants used in the study

No.	Local Name	Scientific Name	Plant Family	The Part Used
1	Clove	*Syzygium aromaticum*	Caryophyllaceae	Flowers
2	Cinnamon	*Cinnamomum zeylanicum*	Lauraceae	Phloem
3	Bay	*Laurus nobilis*	Lauraceae	Leaves
4	Cumin	*Cuminum cyminum*	Apiaceae	Seeds
5	Anise	*Pimpinella anisum*	Apiaceae	Seeds
6	Wild thyme	*Thymus serpyllum*	Lamiaceae	Flowers

2.2 Isolation and identification of fungi

Fungi (previously identified) were isolated from infected fruits and vegetables on potato dextrose agar (PDA) medium after surface sterilization of the tissues with 1% sodium hypochlorite solution. The isolates were purified, and the isolated fungi were observed microscopically based on the morphological characteristics of the spores and conidia, and using the taxonomic keys of research [9].

2.3 Effect of extracts on radial growth

The poisoned food technique was used to test the effect of extracts on radial growth. Different concentrations of each extract were added to sterile PDA medium before solidification. The plates were inoculated with a fungal disc (5 mm) and incubated at 27 ± 2 ℃. The adjusted colony diameter (cm) was measured at full growth in the control treatment, and the percentage inhibition was calculated according to the equation:

$$\text{Inhibition (\%)} = \frac{d_c - d_t}{d_c} \times 100$$

where, d_c = colony diameter in the control; d_t = colony diameter in the treatment.

2.4 Effect of extracts on biomass: Fresh and dry weight

The experiment was performed in potato dextrose broth medium (PDB) following research [10] with some modifications. A total of 600 mL of medium was aliquoted into six 200 mL Erlenmeyer flasks, with 100 mL of PDB per flask. Plant extracts were added to each flask to achieve a final concentration of 100%, after which the flasks were inoculated with one mycelial plug and incubated at 28 ℃ for 14 days (pH 6.7). The experiment was repeated twice. After incubation, the fresh and dry weights were measured by balance:

Fresh weight: Mycelium was harvested by filtration using filter paper No. 1, washed with distilled water, and surface-dried using blotting paper prior to measurement [11].

Dry weight: Mycelium was oven-dried at 60 ℃ for 24 hrs. until a constant weight was reached, and the dry weight was recorded in milligrams [12].

2.5 In vivo tomato fruit protection assay

An in vivo test was performed to evaluate the protective efficacy of the extracts on tomato fruits (*Solanum lycopersicum*) according to study [13]. Healthy fruits were superficially sterilized and then immersed in the plant extract (optimum concentration) for 5 minutes. After drying, a 2 mm deep wound was made where the fruits were inoculated with a spore suspension (1×10^6 spores/ml). The fruits were incubated in humid chambers at 25 ℃. The severity of infection was assessed by measuring the rot rate after 7 days.

A special scale was employed for the disease index, comprising four degrees of disease severity [14]:

0 = Healthy fruit

1 = Fruits covered by rot at a rate of 1–25% of their surface area

2 = Fruits covered by rot at a rate of 26 – 50% of their surface area

3 = Fruit covered by rot at a rate exceeding 50% of its area

The percentage of infection severity was calculated according to the following equation:

$$\text{Infection severity } (\%) = \frac{(n_0\times0)+(n_1\times1)+(n_2\times2)+(n_3\times3)}{N\times3}\times 100$$

2.6 Statistical analysis

Data were statistically analyzed using analysis of variance (ANOVA) according to a completely randomized design (CRD). Mean values were compared using the least significant difference (LSD) test at the 0.05 level of significance.

The Statistical Packages of Social Sciences [15] program was used to test the effect of group differences on the study variables. LSD was used to compare the level of significance of the difference between means.

3. RESULTS

3.1 Fungi isolated from fruits and vegetables

Fungi were isolated from fruits and vegetables, purified, and identified based on morphological characteristics on culture plates and microscopic characteristics. The results revealed various species of fungi, such as *Aspergillus niger*, *Rhizopus stolonifer*, *Fusarium oxysporum*, and *Penicillium expansum* (Figure 1).

3.2 Effect of the plant extracts on radial growth of some isolated fungi

Table 2, Figure 2, and Figure 3 show a significant effect of extract concentrations on the growth diameters of the studied fungi (*A. niger*, *R. stolonifer*, *P. expansum*, and *F. oxysporum*). A gradual decrease in the average growth diameter was observed with increasing extract concentration compared to the control treatment (0%). The highest growth diameter values were recorded at a 0% concentration, while higher concentrations, particularly 75% and 100%, led to a clear inhibition of fungal growth. At a 100% concentration, the growth diameters decreased to their lowest levels for all fungi, reaching 31.67 ± 0.88 mm for *A. niger*, 41.33 mm for *R. stolonifer*, 40.00 mm for *P. expansum*, and 30.00 mm for F. oxysporum. The significant differences, represented by different codes within each column, and based on the LSD value at a probability level of ($P \leq 0.05$), indicate that the effect of concentrations was significant among the treatments, confirming the high inhibitory efficacy of the extract with increasing concentration.

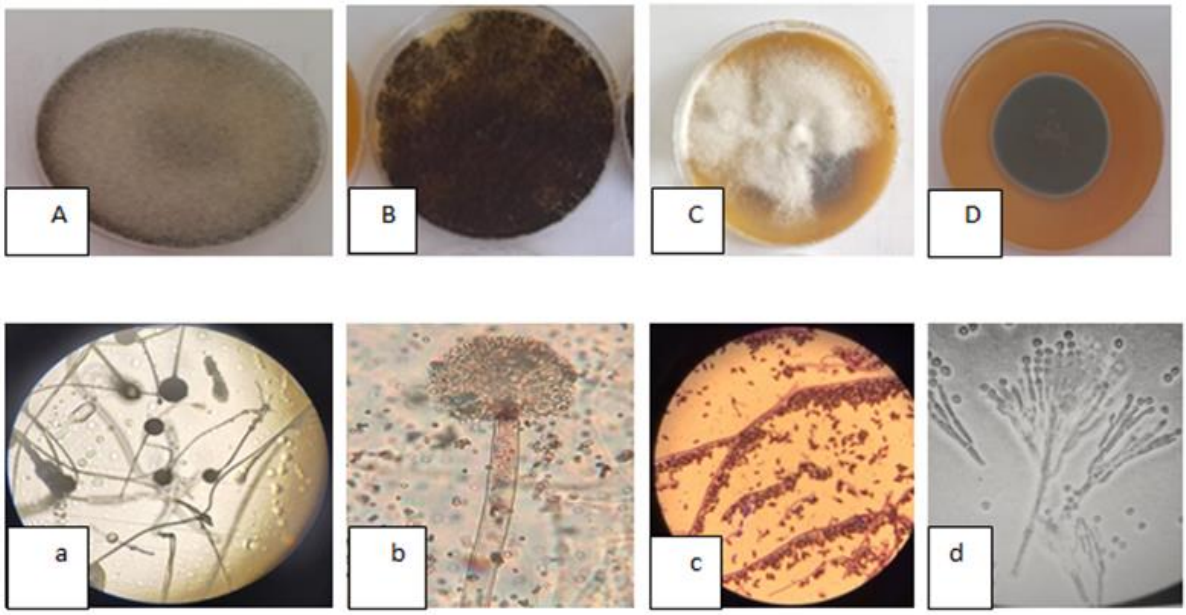

Figure 1. Morphological and microscopical of (A, a) *R. stolonifer,* (B, b) *A. niger,* (C, c) *F. oxysporum,* (D, d) *P. expansum*

Table 2. Effect of the clove concentrations on the diameters of fungi isolated from fruits and vegetables

	The Diameters of Fungi (mm)			
Clove Con. %	*A. niger*	*R. stolonifer*	*P. expansum*	*F. oxysporum*
0	88.33 ± 1.67 a	90.00 ± 0.02 a	82.33 ± 1.45 a	72.67 ± 1.45 a
25	85.00 ± 0.02 b	91.67 ± 1.66 a	80.33 ± 0.33 a	71.00 ± 1.00 a
50	81.00 ± 1.00 c	86.67 ± 0.88 a	76.67 ± 0.88 b	62.67 ± 2.67 b
75	71.67 ± 0.88 d	80.00 ± 2.89 b	57.67 ± 1.45 c	51.33 ± 0.88 c
100	31.67 ± 0.88 e	41.33 ± 0.88 c	40.00 ± 1.15 d	30.00 ± 1.06 d
LSD	3.254 *	5.016 *	3.577 *	4.948 *

Means with the different letters in the same column differed significantly.
* ($P \leq 0.05$).

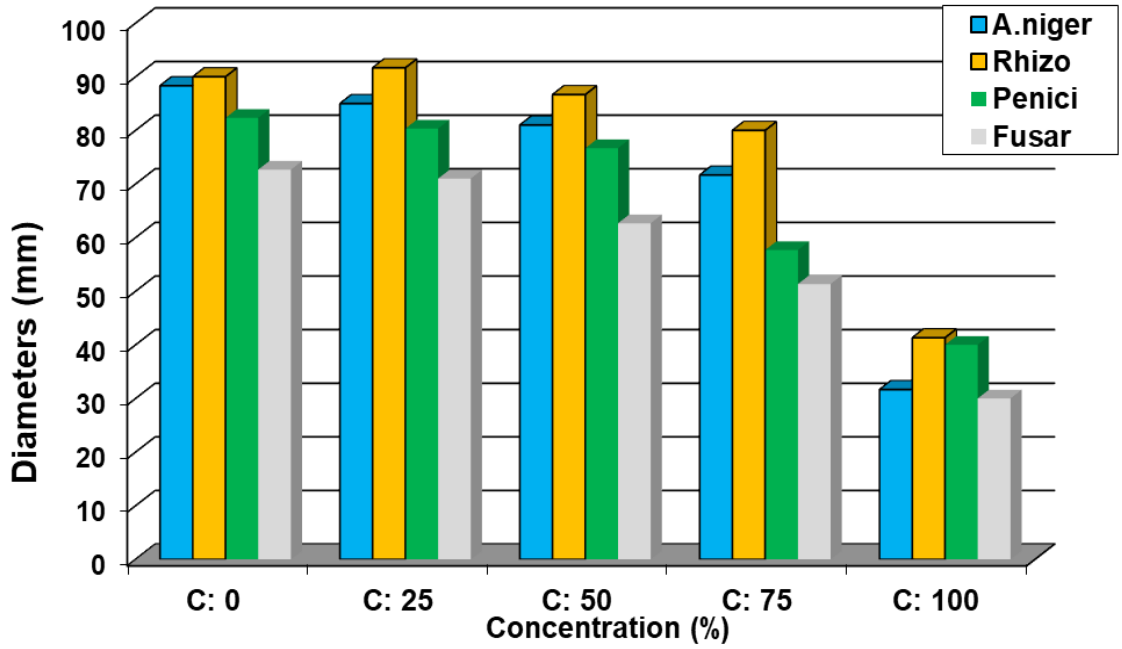

Figure 2. Effect of the clove concentrations on the diameters of fungi isolated from fruits and vegetables

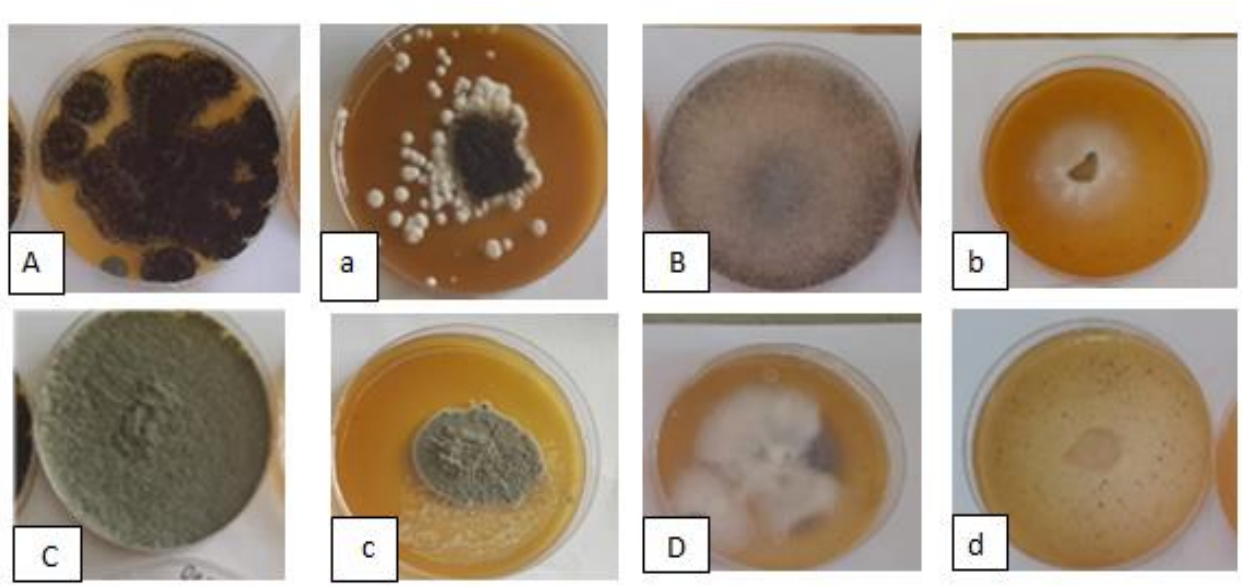

Figure 3. Effect of clove extract (100%) on fungal colony growth: (a) *Aspergillus* (treated) and (A) control; (b) *Rhizopus* (treated) and (B) control; (c) *Penicillium* (treated) and (C) control; (d) *Fusarium* (treated) and (D) control

The results in Table 3, Figure 4, and Figure 5 reveal a clear inhibitory effect of cinnamon concentrations on fungi growth as colony diameters decreased significantly with increasing concentration compared to the control treatment. The greatest decrease in growth was recorded at 100% concentration for all fungi species, with the lowest recorded growth diameter 21.00 ± 0.58 mm on *A. niger*, which raised to 21.66 ± 1.67 mm on *F. oxysporum*, 22.33 ± 1.45 mm on *P. expansam*, and 32.66 ± 1.45 mm on *R. stolonifer*.

The data in Table 4, Figure 6, and Figure 7 clearly demonstrate the effect of thyme concentrations on the growth diameters of fungi isolated from fruits and vegetables. Initially, in the absence of extract (0% concentration), the diameters of the four fungi were at their largest; for example, the average diameter of *A. niger* reached approximately 87.67 ± 1.45 mm, while the average diameter of *Fusarium* scored approximately 75.00 ± 2.89 mm, reflecting vigorous fungal growth in the control medium. While the thyme concentration gradually increased from 25% to 100%, a significant and successive decrease in fungal diameters was observed, indicating an inhibitory effect of the thyme extract on the growth of these fungi species. The most notable suppression was observed at the highest concentration (100%), where the diameter of *A. niger* decreased to approximately 38.33 ± 1.67 mm, and *F. oxysporum* decreased to 15.00 ± 2.51 mm, the lowest value among all parameters. This indicates a strong inhibitory effect of this extract on fungal growth (Table 4). The variations in mean values in the same column indicate statistically significant differences ($P < 0.05$), confirming that the change in diameter across concentrations is not random, but rather correlated with increasing extract concentrations.

Table 3. Effect of the cinnamon concentrations on the diameters of fungi isolated from fruits and vegetables

	The Diameter of Fungi (mm)			
Cinnamon Con. %	*A. niger*	*R. stolonifer*	*P. expansum*	*F. oxysporum*
0	88.00 ± 1.52 a	90.00 ± 0.02 a	80.33 ± 0.33 a	74.00 ± 2.08 a
25	84.33 ± 0.67 b	88.00 ± 0.58 ab	74.67 ± 0.88 b	71.00 ± 0.58 a
50	80.00 ± 0.03 c	83.00 ± 1.53 c	71.67 ± 0.88 b	58.67 ± 4.66 b
75	61.67 ± 1.66 d	55.00 ± 2.89 c	41.66 ± 1.20 c	32.67 ± 1.45 c
100	21.00 ± 0.58 e	32.66 ± 1.45 d	22.33 ± 1.45 d	21.66 ± 1.67 d
LSD	3.419 *	5.102 *	3.220 *	7.89 *

Means with the different letters in the same column differed significantly.
* ($P \leq 0.05$).

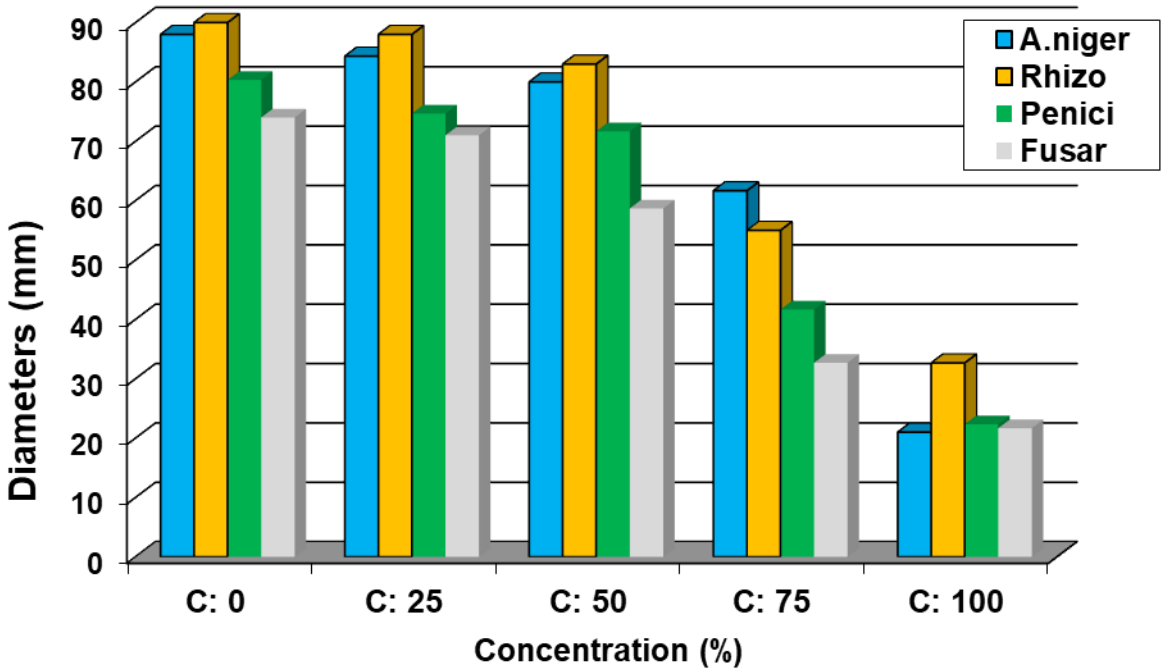

Figure 4. Effect of the cinnamon concentrations on the diameters of fungi isolated from fruits and vegetables

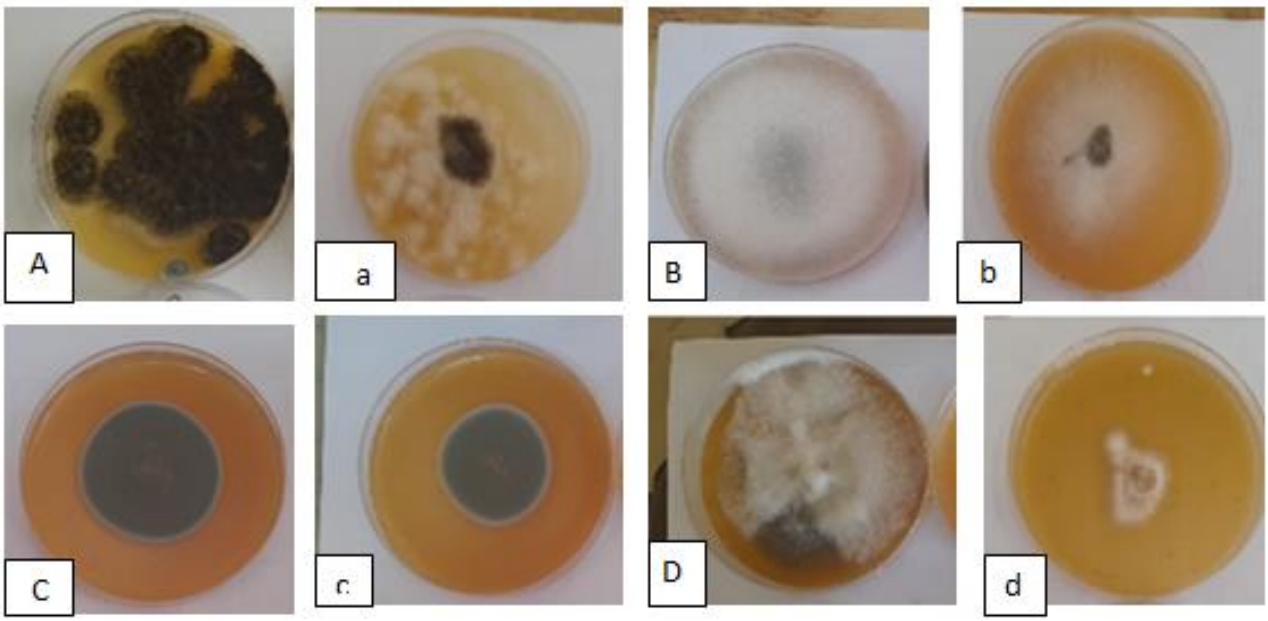

Figure 5. Effect of cinnamon extract (100%) on fungal colony growth: (a) *Aspergillus* (treated) and (A) control; (b) *Rhizopus* (treated) and (B) control; (c) *Penicillium* (treated) and (C) control; (d) *Fusarium* (treated) and (D) control

Table 4. Effect of the thyme concentrations on the diameters of fungi isolated from fruits and vegetables

	The Diameter of Fungi (mm)			
Thyme Con. %	*A.niger*	*R. stolonifer*	*P. expansum*	*F. oxysporum*
0	87.67 ± 1.45 a	90.00 ± 0.00 a	84.33 ± 0.67 a	75.00 ± 2.89 a
25	86.66 ± 0.88 a	90.00 ± 0.02 a	80.00 ± 0.02 b	66.67 ± 3.33 b
50	74.33 ± 1.76 b	85.00 ± 0.04 b	75.00 ± 0.05 c	54.00 ± 2.08 c
75	63.67 ± 1.86 c	80.33 ± 0.33 c	61.00 ± 1.00 d	30.00 ± 0.02 d
100	38.33 ± 1.67 d	80.00 ± 0.02 c	32.67 ± 1.45 e	15.00 ± 2.51 e
LSD	4.926 *	0.469 *	2.657 *	7.732 *

Means having with the different letters in same column differed significantly.
* ($P \leq 0.05$).

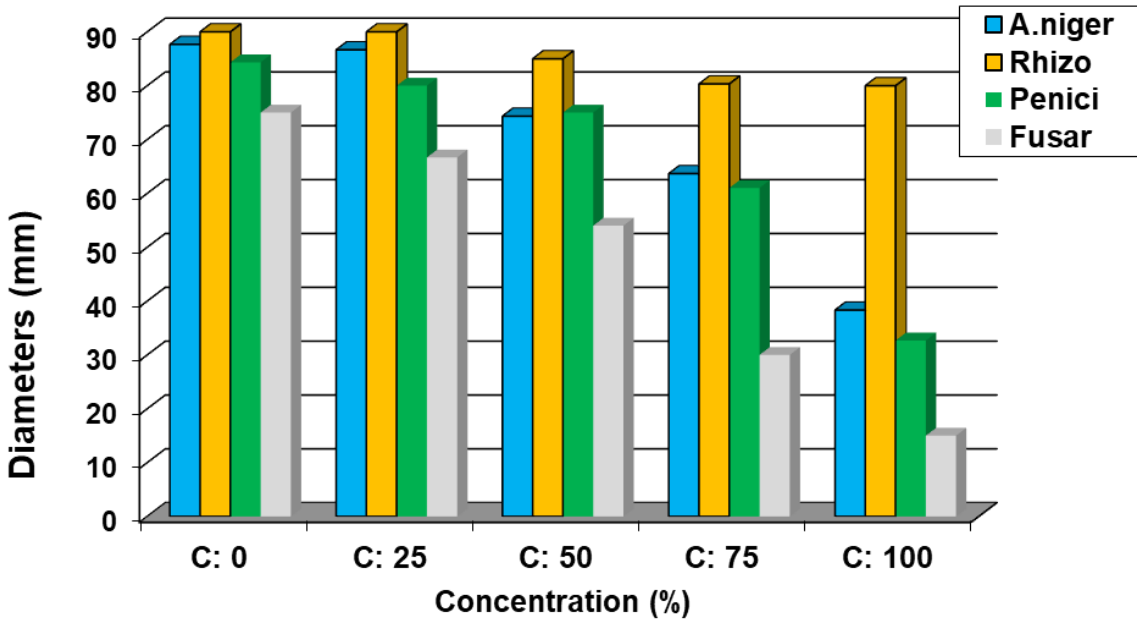

Figure 6. Effect of the thyme concentrations on the diameters of fungi isolated from fruits and vegetables

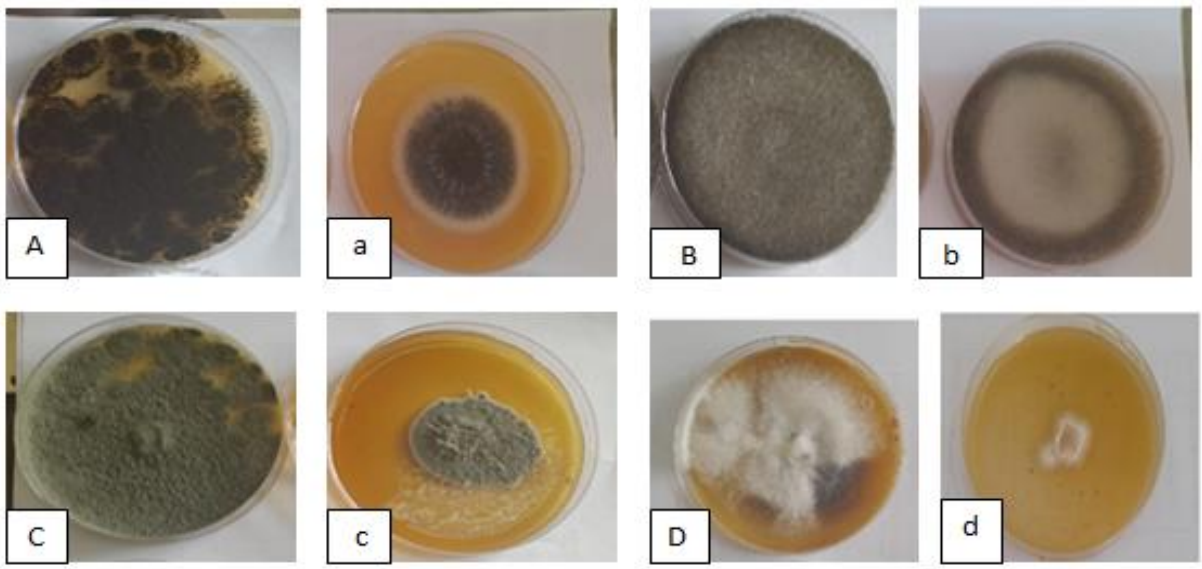

Figure 7. Effect of thyme extract (100%) on fungal colony growth: (a) *Aspergillus* (treated) and (A) control; (b) *Rhizopus* (treated) and (B) control; (c) *Penicillium* (treated) and (C) control; (d) *Fusarium* (treated) and (D) control

Table 5, Figure 8, and Figure 9 show the effect of different concentrations of bay extract on the mycelial growth diameters of four fungal species isolated from fruits and vegetables: *A. niger, R. stolonifer, P. expansum, and F. oxysporum*. The results indicate that the control treatment (0%) exhibited the highest growth diameters for all fungi, ranging from 83.33 ± 1.67 to 90.00 ± 0.02 mm, indicating normal fungal growth in the absence of the extract.

While the bay concentration increased to 25% and 50%, a gradual decrease in the growth diameters of all fungal species was observed, with statistically significant differences in some treatments compared to the control. This decrease continued more clearly at the 75% and 100% concentrations, where the lowest growth diameters were recorded, especially at the 100% concentration, with a growth diameter of 41.33 ± 1.33 mm in *A. niger*, 50.00 ± 2.88 mm in *R. stolonifer*, 23.33 ± 1.66 mm in *P. expansum*, and 33.67 ± 1.86 mm in *F. oxysporum*. The different codes within the same column indicate statistically significant differences at a probability level ($P \leq 0.05$), confirming a clear inhibitory effect of bay extract on the growth of the studied fungi.

Table 5. Effect of the bay concentrations on the diameters of fungi isolated from fruits and vegetables

	The Diameter of Fungi (mm)			
Bay Con. %	*A.niger*	*R. stolonifer*	*P. expansum*	*F. oxysporum*
0	87.67 ± 1.45 a	90.00 ± 0.02 a	83.67 ± 1.86 a	83.33 ± 1.66 a
25	86.67 ± 0.88 a	84.33 ± 0.67 b	78.00 ± 1.00 a	80.33 ± 0.33 a
50	80.33 ± 0.33 b	83.00 ± 0.02 b	61.67 ± 0.88 b	74.00 ± 2.08 b
75	51.67 ± 1.66 c	64.00 ± 2.08 c	50.00 ± 2.88 c	63.67 ± 1.85 c
100	41.33 ± 1.33 d	50.00 ± 2.88 d	23.33 ± 1.66 d	33.67 ± 1.86 d
LSD	3.873 *	5.102 *	5.695 *	5.293 *

Means having with the different letters in same column differed significantly.
* ($P \leq 0.05$).

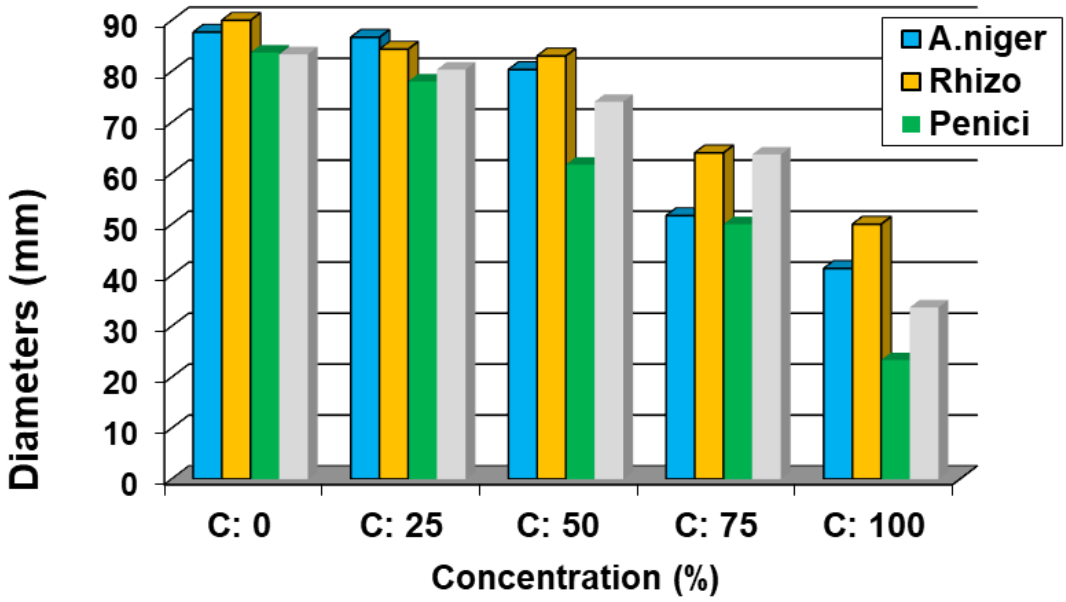

Figure 8. Effect of the bay concentrations on the diameters of fungi isolated from fruits and vegetables

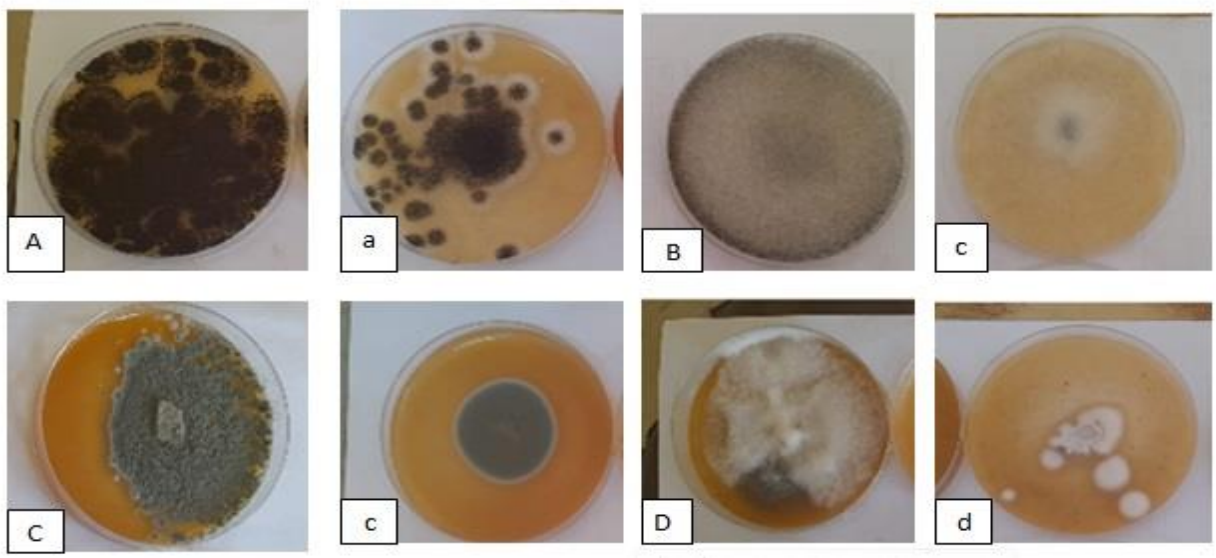

Figure 9. Effect of bay extract (100%) on fungal colony growth: (a) *Aspergillus* (treated) and (A) control; (b) *Rhizopus* (treated) and (B) control; (c) *Penicillium* (treated) and (C) control; (d) *Fusarium* (treated) and (D) control

Table 6. Effect of the anise concentrations on the diameters of fungi isolated from fruits and vegetables

	The Diameter of Fungi (mm)			
Anise Con. %	*A. niger*	*R. stolonifer*	*P. expansum*	*F. oxysporum*
0	88.33 ± 1.67 a	87.33 ± 1.20 a	79.33 ± 2.33 a	76.33 ± 1.33 a
25	87.33 ± 1.20 ab	85.00 ± 0.05 a	78.33 ± 1.67 a	69.00 ± 0.58 b
50	85.00 ± 0.02 b	80.00 ± 0.02 b	71.33 ± 0.88 b	66.00 ± 1.00 bc
75	80.00 ± 0.02 c	75.00 ± 0.02 c	67.66 ± 1.45 bc	62.00 ± 2.00 cd
100	70.00 ± 0.05 d	70.33 ± 2.60 d	62.33 ± 2.33 c	58.33 ± 1.67 d
LSD	2.895 *	4.041 *	5.733 *	4.431 *

Means having with the different letters in same column differed significantly.
* ($P \leq 0.05$).

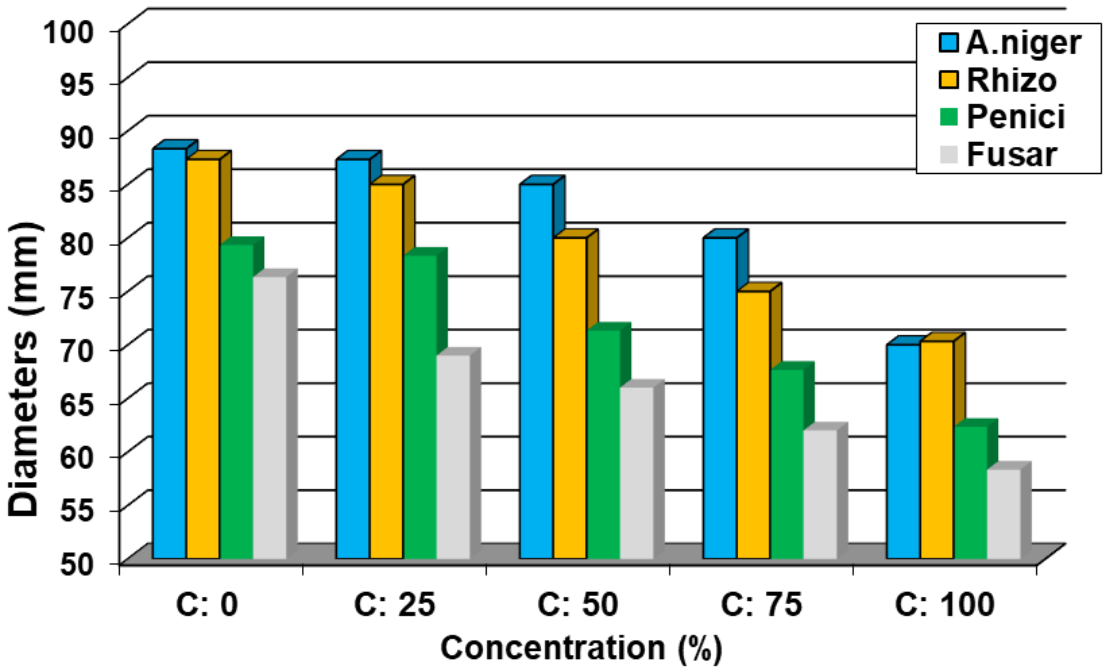

Figure 10. Effect of the anise concentrations on the diameters of fungi isolated from fruits and vegetables

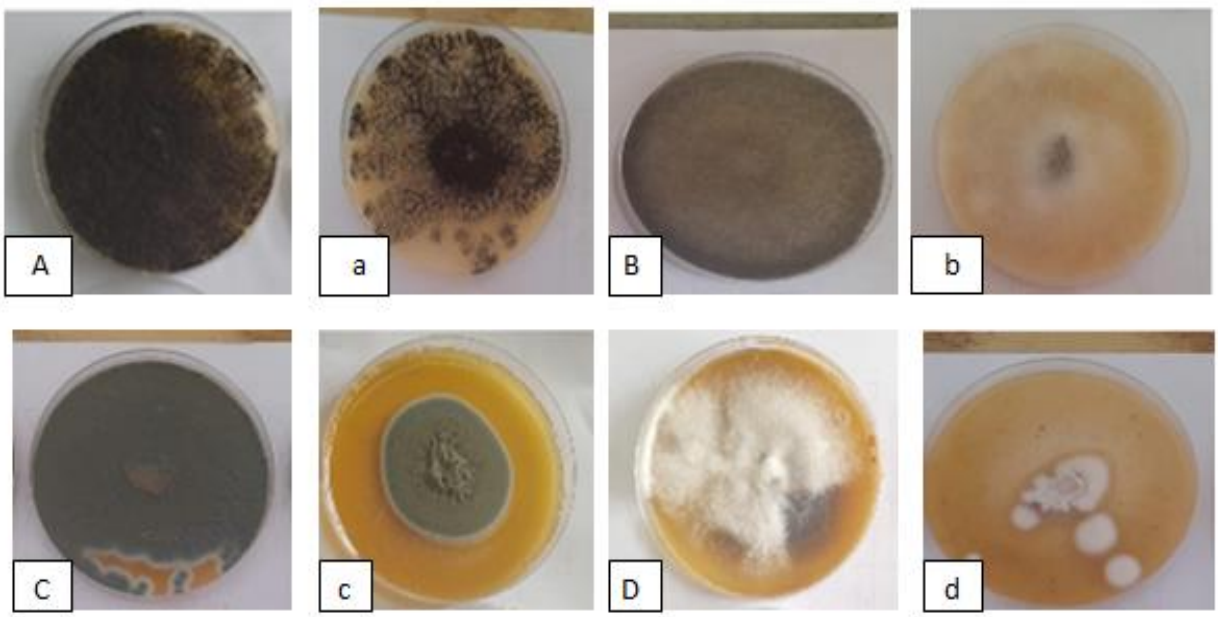

Figure 11. Effect of anise extract (100%) on fungal colony growth: (a) *Aspergillus* (treated) and (A) control; (b) *Rhizopus* (treated) and (B) control; (c) *Penicillium* (treated) and (C) control; (d) *Fusarium* (treated) and (D) control

Table 6, Figure 10, and Figure 11 show the effect of different concentrations of anise extract on the growth diameters of four isolated fruit and vegetable fungi: *A. niger, R. stolonifer, P. expansum, and F. oxysporum.* The results demonstrate that the control treatment (0%) resulted in the highest growth diameters for all fungi, ranging from 76.33 ± 1.33 to 88.33 ± 1.67 mm, indicating normal fungal growth in the absence of the extract.

The anise concentration increased to 25% and 50%, the growth diameters of all fungi decreased gradually, with statistically significant differences in some treatments compared to the control one. This decrease was clearly observed at the 75% and 100% concentrations, where the lowest growth diameters were recorded, particularly at the 100% concentration. The growth diameter reached 70.00 ± 0.05 mm in *A. niger*, 70.33 ± 2.60 mm in *R. stolonifer*, 62.33 ± 2.33 mm in *P. expansum*, and 58.33 ± 1.67 mm in *F. oxysporum*. Different codes within the same column indicate statistically significant differences at a probability level ($P \leq 0.05$), confirming a clear inhibitory effect of anise extract on the growth of the studied fungi.

Table 7. Effect of the cumin concentrations on the diameters of fungi isolated from fruits and vegetables

	The Diameter of Fungi (mm)			
Cumin Con. %	*A. niger*	*R. stolonifer*	*P. expansum*	*F. oxysporum*
0	90.00 ± 0.05 a	90.00 ± 0.05 a	81.00 ± 1.00 a	82.00 ± 1.00 a
25	88.67 ± 0.66 a	90.00 ± 0.05 a	75.00 ± 0.05 b	80.00 ± 0.02 a
50	85.67 ± 0.66 b	87.33 ± 0.33 a	63.00 ± 1.73 c	75.00 ± 0.10 b
75	80.00 ± 0.02 c	81.00 ± 1.00 b	50.00 ± 0.02 d	65.00 ± 0.10 c
100	68.33 ± 1.66 d	71.66 ± 1.66 c	36.67 ± 1.66 e	34.33 ± 2.96 d
LSD	2.698 *	2.779 *	3.668 *	4.406 *

Means having with the different letters in same column differed significantly.
* ($P \leq 0.05$).

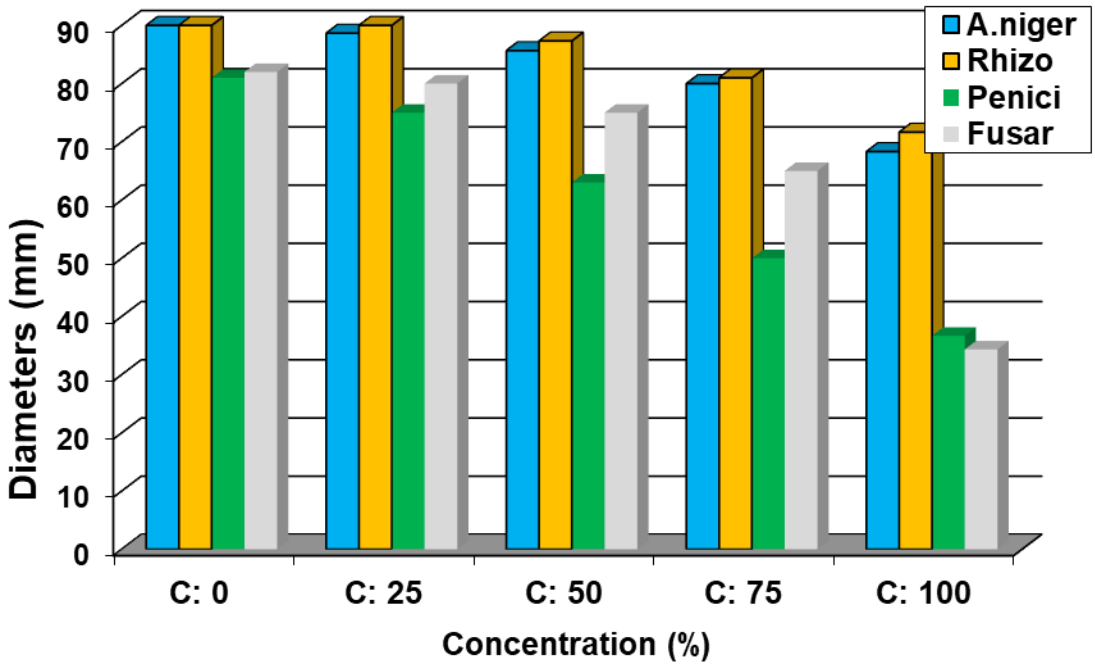

Figure 12. Effect of the cumin concentrations on the diameters of fungi isolated from fruits and vegetables

Table 8. Effect of plant extract concentration (100%) in fresh and dry weight of isolated

Extracts Con. 100%	Fresh Weight (g)				Dray Weight (g)			
	A. niger	*R. stolonifer*	*P. expansam*	*F. oxysporum*	*A. niger*	*R. stolonifer*	*P. expansam*	*F. oxysporum*
Cloves	5.5	6.7	5.9	7.7	3.2	3.3	3.8	3.4
Cinnamon	4.4	5.2	4.9	6.8	2.2	3.1	2.3	3.5
Cumin	6.4	6.5	7.3	6.4	3.2	3.1	3.5	3.1
Anise	8.1	7.8	8.1	7.1	4.4	3.4	5.1	4.3
Bay leaves	8.4	8.1	7.9	7.7	4.3	4.2	3.7	4.4
Wild Thyme	8.3	8.7	8.9	7.6	4.1	3.4	4.5	3.5
Control	8.7	9.1	9.3	8.8	5.1	4.9	5.2	4.7
LSD	2.081 *	2.367 *	3.416 *	1.502 *	1.493 *	1.577 *	1.603 *	1.294 *

* ($P \leq 0.05$).

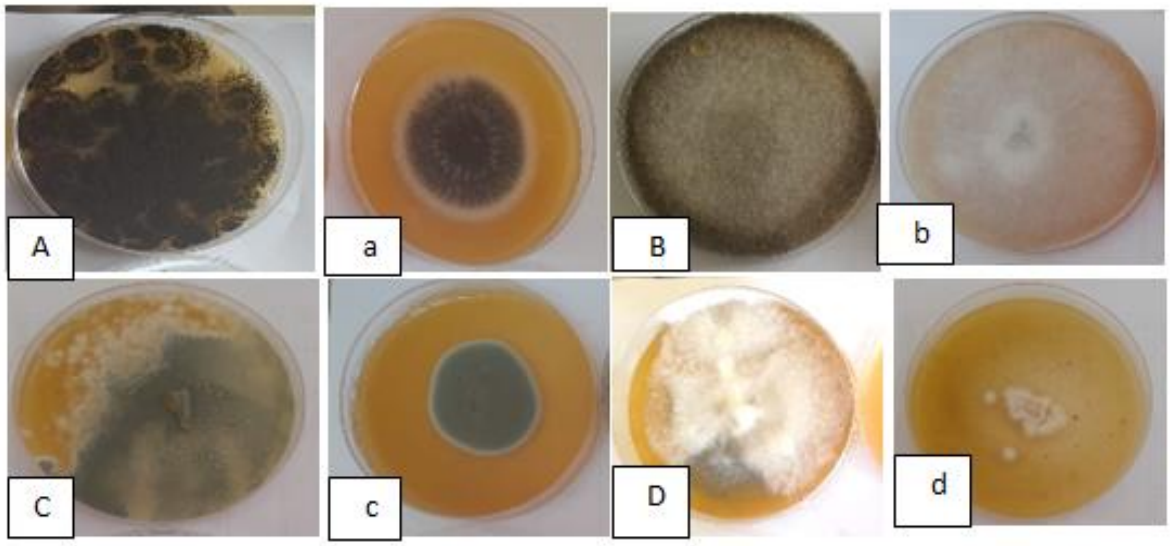

Figure 13. Effect of cumin extract (100%) on fungal colony growth: (a) *Aspergillus* (treated) and (A) control; (b) *Rhizopus* (treated) and (B) control; (c) *Penicillium* (treated) and (C) control; (d) *Fusarium* (treated) and (D) control

The impact of varying cumin extract concentrations on the growth diameters of four separate fungi species—*A. niger, R. stolonifer., P. expansum, and F. oxysporum*—is displayed in Table 7, Figure 12, and Figure 13. The control treatment (0%) produced the largest growth diameters for all fungi, ranging from 81.00 ± 1.00 to 90.00 ± 0.05 mm demonstrating normal fungal growth in the absence of the extract.

All fungi growth diameters gradually decreased as the cumin concentration increased to 25% and 50%, with several treatments showing statistically significant differences compared to the control treatment. The lowest growth diameters were observed at the 75% and 100% concentrations, more clearly at the 100% concentration, which revealed most noticeable decline. *A. niger's* growth diameter was 68.33 ± 1.66 mm, *R. stolonifer* was 71.66 ± 1.66 mm, *P. expansum'* was 36.67 ± 1.66 mm, and *F. oxysporum*' was 34.33 ± 2.96 mm. Anise extract clearly inhibits the growth of the fungi under study; different codes within the same column indicate statistically significant differences ($P < 0.05$).

3.4 Effect of plant extracts on biomass, fresh and dry weight

Table 8 shows the effect of 100% plant extracts on the fresh and dry weight of four isolated fungi (*A. niger, R. stolonifer, P. expansum, and F. oxysporum*). The results showed significant differences at the significance level ($P \leq 0.05$) between the different treatments compared to the control treatment. Bay leafs extract recorded the highest fresh weight values for all fungi, *A. niger* having the highest fresh weight (8.4 g), followed by *R. stolonifer* (8.1 g), indicating a weak inhibitory effect of this extract. In contrast, cinnamon extract showed the lowest fresh weight values, especially for *A. niger* (4.4 g) and *R. stolonifer* (5.2 g), indicating a clear inhibitory effect. Regarding dry weight, anise and cumin extracts recorded relatively high values compared to the other extracts, while cinnamon and clove extracts showed the lowest dry weights, particularly for *P. expansum* and *F. oxysporum* fungi. The control treatment recorded the highest values in both fresh and dry weight for all fungi types, confirming an inhibitory effect of most plant extracts.

3.5 Tomato fruit protection assay by plant extracts (100%) in vivo

Table 9 and Figure 14 present the effect of treatment with aqueous extracts of a number of medicinal plants extract on the severity of fungal infection of tomato fruits contaminated with *A. niger, F. oxysporum., R. stolonifer* and *P. expansum* by comparing the severity of infection before and after treatment with the extracts, in addition to the control treatment.

Table 9. Evaluating the efficacy of the medicinal plant extracts in the reducing tomato fruit infection by contaminating fungi before and after treatment

		Infection Severity %			
Plant Extracts	Treatments	*A. niger*	*F. oxysporum*	*R. stolonifer*	*P. expansum*
Clove	Before	60	55	70	50
	After	30	33	40	20
Cinnamon	Before	65	40	65	40
	After	23	25	23	25
Wild thyme	Before	40	50	60	55
	After	30	35	45	35
Bay	Before	50	40	70	50
	After	30	30	50	30
Anise	Before	40	50	50	60
	After	30	30	35	40
Cumin	Before	55	65	60	70
	After	40	40	35	50
Control, fruits with spore suspension	**Control (0)**	80	70	75	75

The results showed that the pre-treatment severity of fungal infection was high with plant extracts, with infection rates ranging from 40–65% for *A. niger*, 40–65% for *F. oxysporum*, 50–70% for *R. stolonifer*, and 40–70% for *P. expansum,* while the control treatment recorded the highest infection rates of 70–80% for all tested fungi.

A significant post-treatment decrease in fungal infection severity was observed with plant extracts for all studied fungi compared to the pre-treatment state. Infection severity decreased by 23–40% for *A. niger*, 25–40% for *F. oxysporum*, 23–50% for *R. stolonifer*, and 20–50% for *P. expansum*, demonstrating the inhibitory efficacy of the plant extracts used.

Cinnamon and clove extracts reflected the highest effect in reducing the severity of infection after treatment, exhibiting the lowest infection rates compared to the other extracts, while wild thyme, anise, and cumin extracts showed a moderate effect in reducing the severity of fungal infection.

In general, the results confirm that treatment with aqueous extracts of medicinal plants led to a significant decrease in the severity of fungal infection of tomato fruits compared to the pre-treatment state, indicating the effectiveness of these extracts in reducing the development of fungi that cause fruit rot.

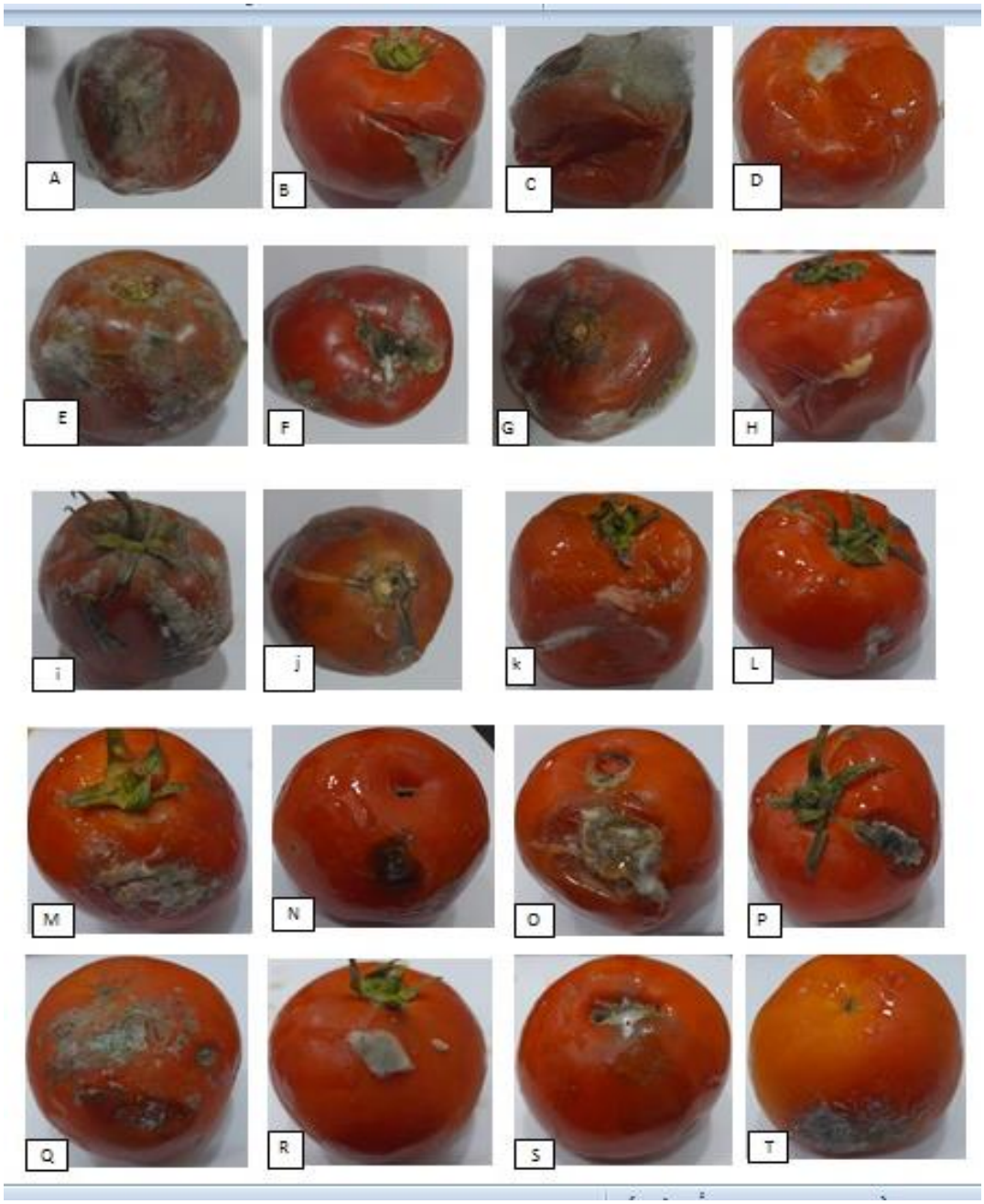

Figure 14. Efficacy of the medicinal plant extracts in the decreasing tomato fruit infection by contaminating fungi before and after treatment with plant extracts

A = tomato infected with *Aspergillus*, B = treated with clove extract then infected with *Aspergillus*; C = tomato infected with *Rhizopus*, D = treated with clove extract then infected with *Rhizopus*; E = tomato infected with *Fusarium*, F = treated with clove extract then infected with *Fusarium*; G = tomato infected with *penicillium*, H = treated with clove extract then infected with *Penicillium*, i = tomato infected with *Aspergillus*, j = treated with cinnamon extract then infected with *Aspergillus*; K = tomato infected with Rhizopus, L = treated with cinnamon extract then infected with *Rhizopus*; M = tomato infected with *penicillium*, N = treated with cinnamon extract then infected with *Penicillium*; O = tomato infected with *Fusarium*, P = treated with cinnamon extract then infected with *Fusarium*; Q = tomato infected with *penicillium*, R = treated with cumin extract then infected with *penicillium*; S = tomato infected with *Fusarium*, T = treated with cumin then infected with *Fusarium*

4. DISCUSSION

Table 2 shows that increasing the concentration of clove extract significantly reduced the growth diameters of all fungi species examined, indicating a concentration-dependent inhibitory effect. The effect was mostly observed at higher concentrations (75% and 100%), where the lowest fungal growth values were recorded. This aligns with study [16] which explained that the antifungal activity of clove increases with increasing concentration of its active phenolic compounds, particularly eugenol; It has good solubility in organic solvents, and moderate solubility in water. This inhibition is attributed to eugenol ability to disrupt fungal cell membrane integrity and influence the activity of vital enzymes, thereby impairing fungal growth [17]. This study indicates that the substance eugenol affects the cell membrane and thus affects on the fungus viability, and this is confirmed by our current study through its effect on the diameter of the fungus and its biomass.

A difference in the sensitivity of the studied fungi was also observed, with species, such as *Fusarium* and *A. niger* exhibiting a greater response to higher concentrations. This may be ascribed to variations in cellular structure and defense mechanisms among the fungal species [18]. These results are consistent with recent studies confirming the potential use of clove extracts as natural and relatively safe alternatives to chemical fungicides, especially in the agricultural and food sectors [19].

The results in Table 3 demonstrate that cinnamon concentrations had a clear and significant inhibitory effect on the growth of all fungi isolated from fruits and vegetables. A gradual decrease in colony diameters was observed with increasing cinnamon concentration compared to the control treatment. Results also reveal variation in the fungi response to different cinnamon concentrations, *Fusarium* was the most sensitive, while *Aspergillus niger* exhibited relatively higher resistance. This difference may be attributed to variations in the chemical composition of fungal cell walls, as well as differences in physiological defense mechanisms among fungal species, such as the efficiency of their toxicity pumps and their tolerance to oxidative stress [20]. The antifungal activity of cinnamon is attributed to its content of bioactive compounds, most notably cinnamaldehyde and phenolic compounds, which disrupt cell membrane integrity, inhibit the activity of vital enzymes, and disrupt essential metabolic processes within the fungal cell, ultimately leading to growth inhibition or fungal cell death [21, 22]. The results of this study are consistent with several recent studies that have confirmed the effective role of cinnamon extracts and oils in inhibiting the growth of food spoilage fungi, and their promising role as natural and safe alternatives to traditional chemical pesticides, especially in the post-harvest preservation of fruits and vegetables [23].

The results in Table 4 showed that thyme extract has antifungal properties. The aqueous extract of thyme possesses concentration-dependent antifungal activity, attributed to water-soluble polar compounds such as phenols and flavonoids [24]. Aqueous extracts have relatively limited effectiveness due to the low solubility of the active compounds (such as thymol and carvacrol) in water, while essential oils exhibit a much higher inhibition of fungal growth. The antifungal effect of thyme is attributed to the presence of phenolic compounds, flavonoids, and traces of thymol (even in the aqueous extract, but in lower concentrations). The mechanism of action includes: Disruption of the fungal cell membrane, increased cell permeability, inhibition of vital enzymes, and disruption of metabolic processes. Studies have shown that these effects lead to damage to the fungal cell wall and membrane, and leakage of cell components [25]. Recent studies indicate that the aqueous extract mainly contains: phenolic compounds, flavonoids, tannins, and antioxidants [26].

The results in Table 5 clearly demonstrate an inverse relationship between the concentration of bay extract and the mycelial growth diameters of the fungi, with higher concentrations leading to a significant decrease in growth. This effect is attributed to the presence of bioactive compounds in bay, particularly bay leaves (*Laurus nobilis* L.), such as phenols, and terpenes, which possess antifungal activity. Recent studies have indicated that these compounds can inhibit fungal growth by disrupting cell membrane permeability and affecting essential enzymatic functions within the fungal cell [27, 28]. The results also revealed a difference in the degree of fungal sensitivity to the extract, with *Penicillium* and *Fusarium* appearing to exhibit varying levels of sensitivity. They were more affected by a 100% concentration compared to *A. niger* and *Rhizopus.* This difference is supported by variations in genetic and cellular structure among the fungal species, as well as differences in cell wall thickness and chitin and beta-glucan content, which affect the ability of plant compounds to penetrate fungal cells [29]. The results are consistent with recent studies showing that natural plant oils and extracts are promising alternatives to chemical pesticides in controlling food spoilage fungi, especially those isolated from stored fruits and vegetables. Several studies have confirmed that extracts of *Laurus nobilis* exhibit antifungal activity against *Aspergillus*, *Penicillium*, and *Fusarium* genera at relatively high concentrations [30].

The study results in Table 6 showed that anise extract had a significant inhibitory effect on the growth of the studied fungi. The diameter of the fungal colonies decreased gradually with increasing concentration, indicating a dose-dependent effect. *Fusarium oxysporum* was the most susceptible compared to the other species, while *Aspergillus niger* exhibited relative resistance. This effect is attributed to the presence of active compounds in aniseed, such as phenols and anethole, which damage the cell membrane and increase its permeability, leading to leakage of cell components and growth inhibition. These results are consistent with previous studies that demonstrated the antifungal activity of aniseed extracts, particularly against *Aspergillus* and *Penicillium* species. However, it should be noted that aqueous extracts are less effective than essential oils [31, 32].

The results in Table 7 are consistent with scientific literature confirming that cumin contains a range of bioactive compounds, including phenols, flavonoids, and terpenoids, as well as cuminaldehyde, which is one of the most important compounds responsible for antifungal activity. These compounds work by disrupting cell membrane integrity and increasing its permeability, leading to leakage of cell components and disruption of vital metabolic processes within the fungal cell. Cumin contains active compounds such as cuminaldehyde and terpenoids and exhibits broad antifungal activity [33].

Table 8 shows that the plant extracts examined exhibited varying degrees of effectiveness in inhibiting fungal growth, reflected in the reduced fresh and dry weight of the fungi compared to the control treatment. Because the results of

fungal diameter measurements indicated that a 100% concentration of plant extracts was the most efficient in reducing fungal diameters for all fungi and significantly more so than other concentrations, the experiment was limited to select the most efficient concentration (100%) to determine its effect on the fungal biomass. Cinnamon and clove extract demonstrated the highest inhibitory effect, indicating their broad-spectrum antifungal activity. This effectiveness is attributed to the presence of potent phenolic and terpenoid compounds in these plants, such as thymol, carvacrol, and cinnamaldehyde, which disrupt fungal cell membrane integrity, inhibit cell wall synthesis, and disable enzyme systems responsible for cellular growth [34]. These results go in line with recent studies confirming that plant extracts can reduce the biomass of foodborne pathogenic fungi such as *Aspergillus* and *Fusarium*, both in culture media and food applications [35]. The variation in response among different fungal species may be attributed to differences in cell wall structure, chitin and beta-glucan content, and varying resistance to oxidative stress.

Table 9 clearly shows that cinnamon and cloves produced the lowest dry weight values, indicating the strongest inhibitory effect on biomass accumulation. This can be explained through several biochemical and physiological mechanisms:

1. Contain highly active phenolic compounds (eugenol, cinnamaldehyde)
2. Disrupt cell membranes, causing leakage and death
3. Inhibit metabolic and enzymatic pathways
4. Induce oxidative damage in the cells
5. Exhibit broad-spectrum antimicrobial activity. All these mechanisms lead to a decrease in Cell growth, biomass accumulation, and then a decrease in Dry weight

The results in Table 9 show that the aqueous extracts of medicinal plants had a clear effect in reducing the severity of fungal infection of tomato fruits contaminated with *A. niger*, *F. oxysporum, R. stolonifer*, and *P. expansum*. As the infection rates decreased after treatment with plant extracts compared to the condition before treatment, the control treatment recorded the highest severity of infection for all tested fungi, which agrees with research [36].

Cinnamon extract recorded the highest efficiency in reducing the severity of post-treatment infection, as infection rates decreased significantly compared to pre-treatment, especially against *A. niger* and *R. stolonifer*. This effect is attributed to cinnamon containing cinnamaldehyde, which has antifungal activity by inhibiting fungal hyphae growth and disrupting cell membrane permeability, thus reducing the fungus's ability to spread within fruit tissues [37].

Clove extract also reflected high efficacy, namely against *P. expansum*, with decreasing post-treatment infection severity compared to the pre-treatment state. This is attributed to the presence of eugenol, a compound that damages the fungal cell wall and interferes with essential metabolic processes within the fungal cell, leading to inhibited fungal growth or death. This aligns with the findings of studies [38, 39] with regard to the effective role of plant phenolic compounds in fungal resistance.

The results also show that all the studied plant extracts led to a relative decrease in pre-treatment infection severity compared to post-treatment, despite the fact that the degree of this decrease varied depending on the plant species and the fungus species used. This variation is attributed to differences in the chemical composition of the plant extracts and the varying concentrations of their active compounds, as well as differences in the sensitivity of the studied fungi to these compounds. This goes in line with Tripathi and Dubey [40], who observed that *R. stolonifer* showed resistance to some extracts compared to *Aspergillus niger* and *Penicillium* spp. fungi, as infection rates remained relatively higher after treatment. This may be ascribed to the rapid growth of this fungus and its high ability to form spores, in addition to the nature of its cell wall, which reduces the effect of some plant compounds on it, which is consistent with studies [37, 41].

In general, the results confirm that treatment with aqueous extracts of medicinal plants reduced the severity of fungal infection in tomato fruits compared to the pre-treatment condition. This highlights the possibility of using them as natural alternatives or support to chemical fungicides in post-harvest disease management programs, given that they are relatively safe and environmentally friendly.

5. CONCLUSION

The results of the current study demonstrate that the tested medicinal plant extracts possess promising high inhibitory activity against pathogenic fungal isolates found in local markets. The data show that radial growth inhibition was accompanied by a substantial decrease in fungal biomass (fresh and dry weight), indicating that the active compounds target both hyphal spread and internal metabolic processes and cellular substance synthesis. This explains the significant decrease in dry weight. Furthermore, in vivo tests proved that using these extracts as a protective coating for tomato fruits effectively reduces the severity of infection and limits the development of the mold diameter resulting from artificial infection.

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