

Efficacy of Cinnamon Alcoholic Extract Compared with Selected Biological, Mineral and Chemical Treatments Against *Alternaria alternata* Causing Foliar Spot Disease on Pepper Plants

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Abstract

Foliar spot disease caused by *Alternaria alternata* is an important constraint to pepper production, particularly under protected cultivation where humidity can favor pathogen development. This study evaluated the efficacy of cinnamon alcoholic extract compared with selected biological, mineral and chemical treatments against *A. alternata* associated with foliar spot of pepper. Symptomatic leaves were collected from pepper fields in Salah al-Din Governorate, Iraq, and the causal fungus was isolated, purified and identified by morphological and molecular approaches. The representative isolate MQ-1 was deposited in NCBI under accession number PX682709.1 and showed 100% identity with *A. alternata* isolate NXU-LM1, while pathogenicity screening of the collected isolates identified AH2 as the most virulent isolate for greenhouse evaluation. Under in vitro conditions, cinnamon alcoholic extract was tested at 5, 10 and 15% using the poisoned food technique and compared with *Bacillus subtilis* filtrate, zinc, Othello Top, Caribo and Zoxis treatments. The extract showed the strongest antifungal activity, with 76.53% inhibition at 5% and complete inhibition at 10 and 15%, giving a mean inhibition of 92.18%. Under greenhouse conditions, cinnamon extract reduced disease incidence to 65.55% and disease severity to 0.283 compared with 90.00% and 0.483 in the infected control, respectively. The treatment also maintained chlorophyll content at 47.19 SPAD units and produced the highest yield per plant (624.38 g), slightly exceeding the fungicide treatment. These findings indicate that cinnamon alcoholic extract has both direct antifungal and protective physiological effects and may be integrated with other management components for reducing *A. alternata* foliar spot on pepper plants.

Keywords: *Alternaria alternata*, cinnamon extract, pepper, foliar spot, biological control, disease severity, chlorophyll, yield.

Introduction

Pepper (*Capsicum annuum* L.) is one of the economically important vegetable crops grown for fresh consumption, processing and spice production. The crop is widely cultivated under open-field and protected conditions, and its market value depends on maintaining vigorous vegetative growth, healthy photosynthetic area and high fruit yield. However, pepper productivity is frequently reduced by several biotic stresses, particularly fungal diseases that attack leaves and fruits and reduce the physiological capacity of the plant [1,2].

Among the foliar pathogens of pepper, *Alternaria alternata* has particular importance

because it is a necrotrophic fungus capable of producing leaf spots, chlorosis and necrotic lesions that expand under favorable environmental conditions. Disease development reduces effective leaf area, limits photosynthesis and eventually affects dry matter accumulation and yield. The pathogen may be more problematic under greenhouse or plastic-house conditions, where high humidity and dense canopy growth can enhance spore germination and lesion expansion [2,3].

Accurate diagnosis of the causal agent is essential before evaluating disease management practices. Although colony morphology and conidial characters are useful for identifying *Alternaria* species, molecular confirmation based on the internal transcribed

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spacer (ITS) region provides stronger evidence for species-level confirmation and enables comparison with globally deposited sequences [4-7]. Such combined identification is particularly important when local isolates are used in pathogenicity and management experiments.

Synthetic fungicides are commonly used to suppress foliar diseases, but exclusive dependence on chemical control can increase selection pressure for resistance and raise concerns about residues, environmental safety and production cost. Therefore, the development of safer alternatives or supportive treatments has become a major direction in plant disease management. Botanical extracts are among the most promising options because they contain biologically active compounds that can directly affect pathogen growth and may also support plant defense responses [8-11].

Cinnamon bark (*Cinnamomum zeylanicum*) contains a complex mixture of phenolic compounds, among which cinnamaldehyde is frequently associated with antifungal activity. Previous studies have shown that cinnamon-derived compounds can affect fungal membrane integrity, inhibit mycelial growth and reduce disease development caused by *Alternaria* spp. and other plant-pathogenic fungi [8-10]. Nevertheless, the effectiveness of cinnamon extract depends on extraction method, concentration, pathogen isolate and host conditions, which makes local evaluation necessary.

This study was designed to evaluate cinnamon alcoholic extract within a comparative management context. Therefore, the present study aimed to identify the causal fungus associated with foliar spot of pepper and to compare the antifungal and protective performance of cinnamon alcoholic extract with selected biological, mineral and chemical treatments against *A. alternata* under in vitro and greenhouse conditions.

Materials and Methods

Collection, isolation and identification of the pathogen

Pepper leaves showing brown to dark foliar spots were collected during the 2024-2025 growing season from fields in Salah al-Din Governorate, Iraq. Samples were placed in paper bags and transferred to the plant pathology laboratory. Small pieces were taken from the margin between diseased and healthy tissues, surface sterilized with 1% sodium hypochlorite for two minutes, rinsed three times with sterile distilled water and dried on sterile filter paper. The pieces were placed on potato dextrose agar (PDA) supplemented with chloramphenicol and incubated at 25 ± 2 °C for 3-5 days. Pure cultures were obtained by hyphal-tip transfer. Morphological identification was based on colony characters and microscopic features of conidia and conidiophores using standard mycological keys [1,4,5].

Molecular confirmation and pathogenicity selection

The ITS region of a representative local isolate was amplified and sequenced for molecular confirmation. The obtained sequence was compared with NCBI sequences using BLAST, and the representative isolate MQ-1 was registered in GenBank under accession number PX682709.1. In parallel, six field isolates were screened for pathogenicity using cabbage seed germination and pepper seedling inoculation tests. Isolate AH2 showed the highest pathogenicity response, as indicated by the greatest inhibition of cabbage seed germination and the development of typical foliar spot symptoms on pepper; therefore, AH2 was selected as the virulent isolate for subsequent in vitro and greenhouse experiments. This approach used the molecularly confirmed isolate to support species-level identification of the local *Alternaria* population, whereas AH2 was selected according to pathogenicity performance for treatment evaluation.

Preparation of cinnamon alcoholic extract

Dried cinnamon bark was obtained from local markets, cleaned and ground into fine powder. The powder was extracted with 96% ethanol by maceration with continuous agitation for 24 h. The mixture was filtered through sterile

gauze and filter paper, then the solvent was allowed to evaporate under aseptic conditions until a crude extract was obtained. The extract was kept in sterile containers at 4 °C until use [12].

In vitro antifungal assay

The poisoned food technique was used to test the inhibitory effect of cinnamon alcoholic extract and other treatments on the mycelial growth of *A. alternata* [13]. Cinnamon extract was incorporated into PDA at 5, 10 and 15%. The comparison treatments included *Bacillus subtilis* filtrate at 5, 10 and 15%, Zn Engie at 2, 4 and 6 mL/L, Zoxis at 0.5, 0.75 and 1.0 mL/L, Othello Top 32.5% SC at 0.5, 0.75 and 1.0 mL/L, and Caribo at 1.5, 2.0 and 3.0 mL/L. A 5-mm disc from the edge of a seven-day-old *A. alternata* culture was placed in the center of each plate. Control plates contained PDA only. Plates were incubated at 25 ± 2 °C until the control reached the plate margin. Colony diameter was measured as the mean of two perpendicular diameters, and inhibition percentage was calculated as $[(C - T)/C] \times 100$, where C is the colony diameter in the control and T is the colony diameter in the treatment.

Greenhouse experiment and measured traits

The plastic-house experiment was conducted in Al-Dibisah, Tikrit District, Salah al-Din Governorate, Iraq, in a house measuring 50 × 9 m. Two hot-pepper cultivars, Barbarian and Balsam F1, were used. Uniform seedlings at the 4–6 true-leaf stage (mostly five true leaves) were transplanted directly into prepared soil beds; therefore, pots were not used in this experiment. The experiment was arranged as a 2 × 6 factorial in a randomized complete block design (R.C.B.D.) with three replicates. Each experimental unit was 2.5 m long and contained 15 plants established on both sides of a drip-irrigation line at 30-cm spacing. Treatments were randomized within each block for each cultivar. The six treatments were a healthy non-inoculated control, an inoculated untreated control, *Bacillus subtilis* culture filtrate (15%), cinnamon alcoholic extract (5%), Zn Engie

applied at the manufacturer-recommended rate, and Othello Top 32.5% SC applied at the manufacturer-recommended rate.

The virulent isolate AH2 was grown on PDA for 10 days. Conidia were dislodged with sterile distilled water, the suspension was filtered through sterile gauze, and its concentration was adjusted with a haemocytometer to 1×10^6 conidia mL⁻¹; no surfactant was added. Artificial inoculation was performed at three stages: while seedlings were still in trays before transplanting, 3 days after transplanting, and 7 days after transplanting. The suspension was applied with a sterile hand sprayer to complete canopy wetting and runoff, with attention to both the upper and lower leaf surfaces. A fixed volume per plant was not used; instead, the same runoff endpoint was applied uniformly to all inoculated plants. Healthy control plants were sprayed with sterile distilled water. High humidity was maintained around the plants after each inoculation to favor conidial germination and infection.

Following completion of the third inoculation, the foliar treatments were applied uniformly with clean hand sprayers until runoff. Disease incidence and severity were assessed 7 days after treatment application on all 15 plants in each experimental unit. Disease incidence (%) was calculated as: (number of symptomatic plants / total number of examined plants) × 100. Disease severity was scored on a 0–5 scale: 0, no symptoms; 1, 1–3 spots covering approximately 1–10% of the leaf area; 2, 4–7 spots covering approximately 10–20%; 3, 8–12 distinct brown-to-dark spots, sometimes with yellow halos or coalescence; 4, generalized spotting on many leaves with the beginning of leaf fall; and 5, spots on most or all leaves, accompanied by leaf fall and plant wilting. The disease severity index (0–1) was calculated as $\Sigma(n \times v)/(N \times V)$, where n is the number of leaves in each score, v is the corresponding score, N is the total number of examined leaves, and V is the maximum score (5) [14]. Chlorophyll content was measured with a SPAD meter [15], and yield per plant was recorded as g plant⁻¹. The healthy control

was included in the analyses of chlorophyll and yield, whereas only the five inoculated treatments were included in the analyses of disease incidence and severity.

Statistical analysis

Laboratory experiments were arranged in a completely randomized design (C.R.D.) with three replicates. The plastic-house experiment was analyzed as a factorial R.C.B.D. with three blocks. Growth, chlorophyll and yield data comprised two cultivars $\times$ six treatments (36 experimental units), whereas disease incidence and severity comprised two cultivars $\times$ five inoculated treatments (30 experimental units). Data were analyzed using the GLM procedure of SAS software (SAS Institute Inc., Cary, NC, USA). Treatment means were

compared using the least significant difference (L.S.D.) test at $P \leq 0.05$.

Results and Discussion

Molecular analysis confirmed the morphological identification of the representative local isolate MQ-1 as *A. alternata*. The ITS sequence was 532 bp and matched *A. alternata* isolate NXU-LM1 with 100% identity and no gaps (Table 1). This molecular record was used to support species confirmation of the local pathogen population, while the separate pathogenicity screening identified isolate AH2 as the most virulent isolate for the treatment evaluation experiments. Combining molecular confirmation with pathogenicity selection strengthened the reliability of the subsequent management assessment [5-7].

Table 1. Molecular identification of *Alternaria alternata* isolate MQ-1 based on ITS sequence similarity.

Similarity (%)	Country	Global accession	Closest matching fungus	Local accession	Local fungus	No.
100	China	PX495952.1	<i>Alternaria alternata</i> isolate NXU-LM1	PX682709.1	<i>Alternaria alternata</i> isolate MQ-1	1

The in vitro assay showed clear concentration-dependent differences among the tested treatments (Table 2). Cinnamon alcoholic extract was the most effective, with a mean inhibition of 92.18%. At 5%, it restricted colony diameter to 1.76 cm and inhibited growth by 76.53%, whereas 10 and 15% completely suppressed visible mycelial growth. Relative to the 7.50-cm colony in the untreated control, these values demonstrate a strong direct fungistatic and fungicidal-like response under the conditions of the poisoned-food assay. The dose response is consistent with the increasing availability of cinnamon-derived active constituents, particularly cinnamaldehyde, which can alter membrane permeability, disturb cellular homeostasis and

suppress hyphal development in *A. alternata* [8–11].

Among the chemical fungicides, Othello Top ranked second, with a mean inhibition of 82.40%, and its activity increased from 73.73 to 91.60% as the concentration increased from 0.5 to 1.0 mL L⁻¹. Caribo showed intermediate activity, while Zoxis was less effective against the tested isolate. *Bacillus subtilis* filtrate produced 54.98% mean inhibition and Zn Engie produced 41.91%. Their lower performance on artificial medium does not necessarily indicate limited plant protection, because mechanisms such as competition, induced resistance and nutritional support are incompletely represented in an agar assay. Thus, Table 2 primarily distinguishes direct antifungal activity, for

which cinnamon extract showed the clearest and most consistent response.

Table 2. Effect of tested treatments and concentrations on colony diameter and inhibition percentage of *Alternaria alternata* under in vitro conditions.

Mean inhibition of treatment (%)	Inhibition (%)	Colony diameter (cm)	Concentration	Treatment
92.18	76.53	1.76	5%	Cinnamon alcoholic extract
	100.00	0.00	10%	
	100.00	0.00	15%	
34.22	24.40	5.67	0.5 mL/L	Zoxis (Azoxystrobin)
	36.00	4.80	0.75 mL/L	
	42.27	4.33	1.0 mL/L	
82.40	73.73	1.97	0.5 mL/L	Othello Top (Azoxystrobin + Difenconazole)
	81.87	1.36	0.75 mL/L	
	91.60	0.63	1.0 mL/L	
58.98	50.67	3.70	1.5 mL/L	Caribo (Carbendazim)
	58.67	3.10	2.0 mL/L	
	67.60	2.43	3.0 mL/L	
54.98	46.27	4.03	5%	<i>Bacillus subtilis</i>
	53.33	3.50	10%	
	65.33	2.60	15%	
41.91	36.93	4.73	2 mL/L	Zn Engie
	41.73	4.37	4 mL/L	
	47.07	3.97	6 mL/L	
0.00	0.00	7.50	-	Control

L.S.D. ($P \leq 0.05$): interaction = 0.421 cm and 5.61% inhibition; concentration = 0.159 cm and 2.12% inhibition; treatment = 0.243 cm and 3.24% inhibition.

Under plastic-house conditions, disease incidence was highest in the inoculated untreated control (90.00%) and differed among treatments (Table 3). Othello Top and cinnamon alcoholic extract recorded the lowest means, 61.11 and 65.55%, respectively,

and did not differ significantly from each other. These values represent relative reductions of 32.10 and 27.17%, respectively, compared with the inoculated control. Zn Engie reduced incidence to 79.89%, whereas the *B. subtilis* filtrate produced a smaller

numerical reduction to 82.22% and did not separate statistically from the inoculated control at the adopted L.S.D. level. The strong performance of cinnamon extract agrees with its direct inhibition *in vitro* and suggests that active compounds remained effective on the

leaf surface, limiting infection establishment or early lesion development [8–10]. The non-significant cultivar effect indicates that Barbarian and Balsam F1 had comparable susceptibility to AH2 under the imposed infection pressure.

Table 3. Effect of selected treatments and pepper cultivars on disease incidence (%) under greenhouse conditions.

Mean	Balsam F1	Barbarian	Treatment
90.00	88.89	91.11	Infected control
82.22	82.22	82.22	<i>Bacillus subtilis</i> filtrate
65.55	64.44	66.67	Cinnamon alcoholic extract
79.89	82.22	77.56	Zn Engie
61.11	62.22	60.00	Othello Top 32.5% SC
---	76.01	75.51	Mean

L.S.D. ($P \leq 0.05$): cultivar = N.S.; treatment = 8.08; interaction = N.S.

Disease severity followed a clearer treatment gradient (Table 4). The inoculated control recorded the highest index (0.483), while Othello Top reduced it to 0.237, a 50.93% relative reduction. Cinnamon extract ranked second at 0.283, corresponding to a 41.41% reduction, and its severity was significantly lower than those of Zn Engie (0.381) and *B. subtilis* filtrate (0.391). Zinc and the bacterial filtrate did not differ significantly from each other, although both reduced severity relative to the inoculated control. The lower severity

under cinnamon treatment can be explained by restricted hyphal growth and slower lesion expansion after penetration, consistent with membrane-disruptive and growth-inhibitory effects reported for cinnamaldehyde and related cinnamon constituents [8–10]. In contrast, the moderate responses to zinc and *Bacillus* likely reflect mainly host-supporting or induced-defense mechanisms rather than the strong direct inhibition observed for cinnamon on PDA [17–20].

Table 4. Effect of selected treatments and pepper cultivars on disease severity index under greenhouse conditions.

Mean	Balsam F1	Barbarian	Treatment
0.483	0.467	0.500	Infected control
0.391	0.403	0.380	<i>Bacillus subtilis</i> filtrate
0.283	0.283	0.283	Cinnamon alcoholic extract
0.381	0.380	0.383	Zn Engie
0.237	0.240	0.233	Othello Top 32.5% SC
---	0.354	0.356	Mean

L.S.D. ($P \leq 0.05$): cultivar = N.S.; treatment = 0.040; interaction = N.S.

Chlorophyll content provided a physiological measure of treatment protection (Table 5). Infection reduced the mean SPAD value from 55.88 in the healthy control to 36.24 in the inoculated control, confirming substantial impairment of functional leaf tissue. Cinnamon extract gave the highest value among inoculated treatments (47.19), an increase of 30.22% over the inoculated control. It did not differ significantly from Othello Top (44.39) or *B. subtilis* filtrate (44.20), but exceeded Zn Engie (42.35). Preservation of chlorophyll under cinnamon

treatment is consistent with reduced lesion development and retention of a larger photosynthetically active leaf area. Biological and mineral treatments may also help maintain pigment stability by moderating pathogen-induced oxidative stress and supporting defense-related metabolism [16–18]. Barbarian had a higher overall SPAD mean than Balsam F1, indicating a cultivar-dependent difference in physiological performance even though their disease incidence and severity did not differ significantly.

Table 5. Effect of selected treatments and pepper cultivars on chlorophyll content (SPAD units) under greenhouse conditions.

Mean	Balsam F1	Barbarian	Treatment
55.88	53.57	58.18	Healthy control
36.24	34.91	37.57	Infected control
44.20	39.05	49.35	<i>Bacillus subtilis</i> filtrate
47.19	43.84	50.53	Cinnamon alcoholic extract
42.35	39.53	45.17	Zn Engie
44.39	41.18	47.60	Othello Top 32.5% SC
---	42.02	48.07	Mean

L.S.D. ($P \leq 0.05$): cultivar = 2.07; treatment = 3.59; interaction = N.S.

Yield per plant was the most direct agronomic indicator of treatment value (Table 6). Infection reduced yield from 775.37 g plant⁻¹ in the healthy control to 411.29 g plant⁻¹ in the inoculated control. Cinnamon extract produced the highest numerical yield among inoculated treatments (624.38 g plant⁻¹), closely followed by Othello Top (623.31 g plant⁻¹); the difference between them was not significant. Relative to the inoculated control, these responses represented yield increases of 51.81 and 51.55%, respectively. *B. subtilis*

filtrate and Zn Engie also improved yield to 571.31 and 558.97 g plant⁻¹. The four protective treatments did not differ significantly from one another at the treatment L.S.D., but each maintained substantially greater productivity than the inoculated control. Cinnamon therefore combined effective disease suppression with chlorophyll preservation and fruit production, while Barbarian produced a significantly higher overall yield than Balsam F1.

Table 6. Effect of selected treatments and pepper cultivars on yield per plant (g plant⁻¹) under greenhouse conditions.

Mean	Balsam F1	Barbarian	Treatment
775.37	642.10	908.70	Healthy control
411.29	379.00	443.60	Infected control
571.31	473.70	668.90	<i>Bacillus subtilis</i> filtrate
624.38	535.40	713.40	Cinnamon alcoholic extract
558.97	496.10	621.80	Zn Engie
623.31	559.80	686.80	Othello Top 32.5% SC
---	514.35	673.87	Mean

L.S.D. ($P \leq 0.05$): cultivar = 46.36; treatment = 80.30; interaction = N.S.

Taken together, the results show that cinnamon alcoholic extract was effective at two complementary levels. Its complete suppression of visible mycelial growth at 10 and 15% demonstrated strong direct antifungal activity, while the plastic-house responses showed that activity was retained under plant conditions. Cinnamon was statistically comparable with Othello Top for disease incidence, chlorophyll content and yield, although Othello Top produced a significantly lower severity index. This distinction indicates that management value should be judged through both pathological and agronomic traits, rather than through lesion measurements alone.

The relative rankings also reflected different modes of action. Cinnamon extract and the fungicide expressed strong direct effects on the pathogen, whereas *B. subtilis* and zinc showed more moderate disease suppression but still improved chlorophyll and yield. *Bacillus*-based treatments can act through antibiosis, competition and induced systemic resistance [19,20], while zinc contributes to membrane integrity, antioxidant balance and defense-associated metabolism [17,18]. These mechanisms can support plant performance

even when direct inhibition on culture medium is limited.

Cultivar responses further support this interpretation. Barbarian generally maintained higher chlorophyll and yield than Balsam F1, but the two cultivars did not differ significantly in disease incidence or severity, and cultivar $\times$ treatment interactions were not significant for the reported plastic-house variables. Accordingly, the treatments acted consistently across both cultivars, while inherent growth and productivity differences accounted for the superior agronomic performance of Barbarian.

From an integrated disease-management perspective, cinnamon extract should be viewed as a promising botanical component rather than an unconditional replacement for fungicides. Preventive application, rotation with effective fungicides, sanitation and improved ventilation may reduce repeated chemical use while maintaining disease control. Field-scale validation is still needed to standardize extraction, confirm crop safety, define spray intervals and evaluate performance under different environmental and disease-pressure conditions.

General discussion and practical implications

The comparative design strengthens the evidence for cinnamon because it was evaluated beside biological, mineral and chemical treatments rather than in isolation. Its laboratory superiority, coupled with low disease values and high chlorophyll and yield under plastic-house conditions, indicates a reproducible response across distinct assessment levels. The results also emphasize that treatments with different modes of action should be compared through an integrated set of pathogen, physiological and productivity variables.

Othello Top gave the lowest disease incidence and severity, whereas cinnamon produced the highest numerical yield among inoculated treatments. Because cinnamon and Othello Top were statistically similar for incidence, chlorophyll and yield, the botanical treatment offered a balanced response rather than superiority in every single variable. Such balance is important for practical disease management, where protection of productive leaf area and yield can be as consequential as the numerical reduction of lesions.

The moderate performance of *B. subtilis* filtrate and Zn Engie is compatible with their indirect or host-mediated roles. Their effects may become more evident through repeated application, optimized formulations or earlier preventive use. By contrast, cinnamon extract displayed both strong direct inhibition and plant-level protection, explaining its consistent position among the best treatments.

Chlorophyll preservation and yield recovery provide practical evidence that cinnamon treatment limited the physiological consequences of infection. The extract did not restore plants to the healthy-control level, but it substantially narrowed the gap between infected and healthy plants. This result supports its inclusion in integrated programs aimed at reducing disease damage while sustaining pepper productivity.

Further studies should determine the chemical profile and stability of the extract, quantify

any phytotoxicity threshold, optimize application intervals and test performance under open-field conditions. These steps are necessary before a standardized cinnamon-based treatment can be recommended for commercial use.

Conclusion

The representative local causal agent of pepper foliar spot was confirmed as *Alternaria alternata* isolate MQ-1 by morphological and ITS-based molecular identification, while isolate AH2 was selected as the virulent isolate for pathogenicity and greenhouse evaluation. Cinnamon alcoholic extract exhibited the strongest direct antifungal activity among the tested treatments, achieving complete inhibition of mycelial growth at 10 and 15% under in vitro conditions. Under greenhouse conditions, the extract reduced disease incidence and severity, maintained chlorophyll content and produced the highest yield among treated infected plants. Although Othello Top gave the lowest disease incidence and severity, cinnamon extract showed a balanced protective performance by combining disease reduction with improved physiological and productive responses. Therefore, cinnamon alcoholic extract can be considered a promising botanical treatment for inclusion in integrated management of *A. alternata* foliar spot on pepper plants.

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