J. Trop. Plant Pests Dis.
Vol. 26, No. 1, March 2026
Pages: 270–279

ISSN: 1411-7525
E-ISSN: 2461-0399
DOI : 10.23960/j.hptt.126270-279

RESEARCH PAPER

Efficacy of organic and inorganic treatments against Alternaria leaf spot disease of *Conocarpus erectus* L. in Iraq

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Manuscript received: 12 June 2025. Revision accepted: 13 October 2025. Available online: 12 March 2026.

ABSTRACT

Severe leaf spot disease has recently been observed on *Conocarpus erectus* (buttonwood) trees in Iraq, with artificial inoculation resulting in a disease severity of 91.6%. Morphological, microscopic, and molecular analyses using ITS1/ITS4 primers identified *Alternaria alternata* as the causal agent. Two isolates (Alt.1IQ and Alt.2IQ; GenBank accession numbers OM994386 and OM994387) exhibited 98.05–100% sequence similarity with previously reported Iraqi isolates. In vitro antifungal assays demonstrated that magnesium sulphate, sodium bicarbonate, and neem oil at a concentration of 0.4% inhibited fungal growth by 57.63%, 55.62%, and 50.80%, respectively. Laboratory leaf assays revealed that all tested treatments at 0.2–0.4% completely suppressed fungal development, resulting in 0% affected leaf area compared with 31.96% in the untreated control. Nursery trials showed that neem oil and sodium bicarbonate were the most effective treatments, reducing disease severity to 8.3–16.6% and 8.3–25.0%, respectively, while magnesium sulphate achieved a moderate reduction (24.33–25.0%. The chemical fungicide Coastine 38WG completely prevented disease development (0% severity), whereas untreated seedlings exhibited 91.6% disease severity. Overall, neem oil and sodium bicarbonate at 0.4% emerged as promising eco-friendly alternatives for managing *A. alternata* –induced leaf spot disease in *Conocarpus* seedlings. These findings support their potential incorporation into sustainable and integrated disease management strategies aimed at reducing reliance on synthetic fungicides.

Keywords: *Alternaria alternata*, leaf spot, neem oil, sodium bicarbonate, sustainable disease management

INTRODUCTION

Buttonwood trees (*Conocarpus erectus* L.) are small evergreen harsh environmental, its tolerance of high temperatures, poor drainage, and air-polluted conditions (Lonard et al., 2021). Buttonwood trees have diverse applications, including charcoal production, construction materials, and the remediation of oil-contaminated soils (Jawaid et al., 2021). Rich in bioactive compounds such as glycosides, alkaloids, phenols, tannins, and fatty acids like arachidic, stannic, and nonadecanoic acids, *Conocarpus* species have also been recognized for their medicinal properties, particularly their antimicrobial activity (Bashir et al., 2015; Rehman et al., 2019).

Despite their beneficial attributes, *Conocarpus* plants are vulnerable to various diseases, including fungal infections caused by pathogens such as *Alternaria* spp. This genus is known for its broad host range, affecting cereals, vegetables, trees, ornamentals, and oil crops (Thomma, 2003). *Alternaria* species produce toxins that facilitate plant tissue invasion and infection (Saeed & Juber, 2016). These fungi are ubiquitous, being found in soil, air, and water, which makes them a persistent threat to plant health (Alkhail, 2005; Quayyum et al., 2005).

Various control methods have been employed to manage fungal pathogens, with chemical interventions being the most common. However, the extensive use of fungicides raises concerns regarding pathogen resistance and environmental impact (Al-Waily et al., 2018; Othman & Kakey, 2021). Recent studies have demonstrated the potential of biocontrol strategies against *Alternaria*-induced leaf spot diseases. For example, Wati et al. (2024) reported that phyllospheric actinomycetes isolated from Liliaceae effectively suppressed purple blotch disease caused by *Alternaria porri* on shallot, highlighting the feasibility of alternative, environmentally friendly approaches for managing *Alternaria*-associated foliar diseases. Although the host plant and pathogen species differ, these findings support the broader applicability of non-chemical control strategies against *Alternaria* spp.

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Among plant-derived products, neem oil, obtained from *Azadirachta indica*, is widely recognized for its antimicrobial properties. Studies have demonstrated its efficacy in inhibiting fungal growth; for instance, Mossini et al. (2009) reported significant inhibition of *Penicillium* spp. at concentrations of 0.25%, 0.5 % and 1.25%. Similarly, Rodrigues et al. (2019) found that neem oil achieved a 95% inhibition rate against *Aspergillus carbonarius* at a concentration of 0.3%.

In addition to plant extracts, inorganic salts have shown promise in controlling fungal pathogens and inducing plant resistance. Kolaei et al. (2012) observed that magnesium sulfate at a concentration of 2% effectively reduced potato dry rot caused by *Fusarium sambucinum*. Similarly, Türkkan & Erper, (2014) highlighted the role of sodium bicarbonate in inhibiting *Fusarium oxysporum*, with inhibition rates of 51.09%, 74.37%, and 100% at concentrations of 10 mM, 25 mM, and 50 mM, respectively.

Given the widespread cultivation of *Conocarpus* trees in Iraq since 2003, particularly as an ornamental plant in parks, public gardens, and city entrances, the recent emergence of leaf spot disease poses a significant threat. The objective of this study was to assess the effectiveness of selected organic and inorganic compounds with distinct modes of action in managing *Alternaria* leaf spot disease on *Conocarpus,* as well as to identify the isolates under investigation using morphological and molecular methods. This study represents an important step toward developing effective and sustainable strategies to reduce fungal diseases affecting *Conocarpus* trees in arid environments.

MATERIALS AND METHODS

Research Site. This research carried out at the laboratories of the Department of Plant Protection, College of Agricultural Engineering Sciences, University of Baghdad, Iraq, during the growing season of 2024.

Isolation and Identification of Fungi. Samples of *Conocarpus* plants exhibiting symptoms of leaf spot disease were collected from various locations in Baghdad (33.31 °N, 44.36 °E), Iraq (Figure 1). The samples were carefully placed in sterile polyethylene bags (30 × 20 cm) and transported to the laboratory for pathogen isolation.

For pathogen isolation, 0.5 cm segments were excised from both symptomatic and adjacent asymptomatic areas of leaves and branches. Surface sterilization was performed by immersing the segments in a 1% sodium hypochlorite solution for two min, followed by three rinses with sterile distilled water. The segments were then dried on sterile filter paper.

The sterilized plant segments were transferred to plastic Petri dishes (90 mm) containing potato dextrose agar (PDA) medium. The PDA medium (HIMEDIA, India) was prepared by dissolving 39 g of the medium in 1 L of distilled water and sterilized by autoclave at 121 ºC and 1.5 kg/cm² pressure for 15 min. To prevent bacterial contamination, streptomycin sulfate (Agrept 20%, Agrochem co., Egypt) was added to the medium at a concentration of 50 mg/L.

Four plant segments were placed in each Petri dish, with three replicates prepared for each sample. The plates were incubated at 25 ± 2 ºC for three days. Emerging fungal colonies were purified using the single-spore method and identified based on morphological characteristics (Kareem et al., 2020b).

Pathogenicity Test. The pathogenicity of the fungal isolates was evaluated on *Conocarpus* leaves under laboratory conditions. Leaf samples were obtained from the sub-apical region of two-year-old seedlings. Healthy *Conocarpus* leaves were surface sterilized

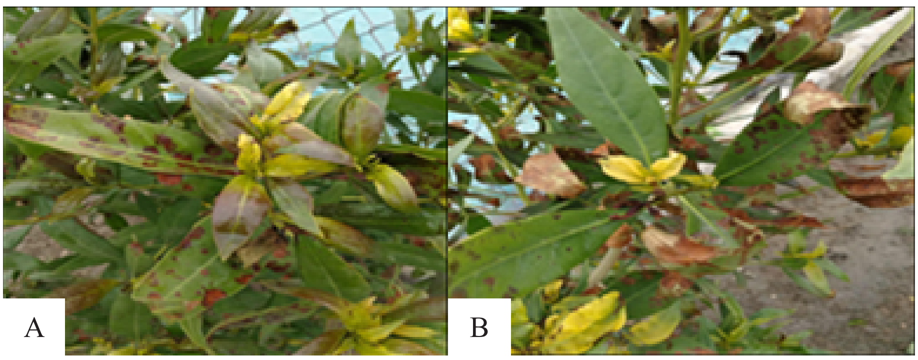

Figure 1. Leaf spot disease symptoms on *Conocarpus* leaves. A, B. Natural symptoms of leaf spot disease on *Conocarpus* leaves.

using a 1% sodium hypochlorite solution (containing 1% free chlorine) for 2 min, followed by rinsing with sterile distilled water. The leaves were then air-dried and placed in Petri dishes lined with three sterile filter papers moistened with distilled water.

A 0.5 cm mycelial disc taken from the margin of a 5-day-old fungal colony was placed at the center of each leaf. Six replicates were prepared for each isolate. The Petri dishes were incubated at 25 ± 2 ºC for 10 days, with the filter papers remoistened as needed. The affected leaf area was calculated using the method described by Ahmed et al., 2025.

Genomic DNA Extraction. Genomic DNA was extracted from the two fungal isolates, Alt.1IQ and Alt.2IQ, identified as *Alternaria* spp. The fungal mycelia were cultivated on PDA medium for 7 days at 25 ± 2 ºC. Mycelia were collected and ground into a fine powder using liquid nitrogen. Genomic DNA was extracted using the NucleoSpin® Microbial DNA kit (Macherey-Nagel GmbH, Germany) according to the manufacturer's protocol.

DNA Amplification and Sequencing. The DNA of *Alternaria* spp. was amplified using polymerase chain reaction (PCR) in a Mastercycler® X50 thermal cycler (Eppendorf AG, Hamburg, Germany). Identification of *Alternaria* was confirmed using the ITS1 (5′-TCCGTAGGTGAACCTGCGG -3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC -3′) primers (Kareem et al., 2020a).

The PCR protocol consisted of an initial denaturation step at 94 ºC for 90 s, followed by 35 cycles of denaturation at 94 ºC for 30 s, annealing at 55 ºC for 30 s, and extension at 72 ºC for 30 s. A final extension step was performed at 72 ºC for 10 min.

The PCR products were sent to Macrogen Corporation (South Korea) for sequencing. The resulting sequences were deposited in the NCBI database (Kareem et al., 2020a). A phylogenetic tree was constructed using the MEGA7 software based on the maximum likelihood method with the Kimura 2-parameter model (Kimura, 1980). The neighbor-joining method was applied to determine genetic distance between isolates using a Maximum Composite Likelihood (MCL) approach (Kumar et al., 2016).

Inhibition of Fungal Pathogens by Organic and Inorganic Compounds. The antifungal activity of neem oil, sodium bicarbonate, and magnesium sulfate against two fungal isolates was evaluated *in vitro* using the poisoned food technique. Potato dextrose agar (PDA) medium amended with each treatment at concentrations of 0.1%, 0.2%, and 0.4% was autoclaved at 121 ºC (1.5 kg/cm^2) for 15 min. Prior to solidification, streptomycin sulfate (50 mg/L) was aseptically added to prevent bacterial contamination.

Each Petri dish was centrally inoculated with a 5 mm mycelial disc taken from the actively growing margin of a 5-day-old fungal colony. The experiment was arranged in a completely randomized design (CRD) with three replicates per treatment, including an control. Colony diameters were measured, and the percentage of mycelial growth inhibition (MGI%) was calculated according to Masoud et al. (2022), using the following formula:

$$\text{MGI\%} = \frac{R - r}{R} \times 100$$

MGI% = Mycelial growth inhibition percentage;
R = Mycelial growth in the control plate;
r = Mycelial growth in the treatment plate.

Efficacy of Botanical and Chemical Compounds Against Leaf Spot Disease. Based on the *in vitro* results, the efficacy of neem oil, sodium bicarbonate, and magnesium sulfate at concentrations of 0.1%, 0.2%, and 0.4%, along with the fungicide Coastine 38WG (pyraclostrobin 12.8% + boscalide 25.2%) applied at the manufacturer's recommended dose, was evaluated on *Conocarpus* leaves using a detached-leaf assay.

Leaves were surface-sterilized with 1% sodium hypochlorite (1% free chlorine), rinsed with sterile distilled water, and air-dried under aseptic conditions. The sterilized leaves were placed in Petri dishes lined with three layers of sterile filter paper moistened with sterile water. A 0.5 cm mycelial disc taken from the edge of a 5-day-old fungal colony grown on PDA was placed at the center of each leaf.

The dishes were incubated at 25 ± 2 ºC for 24 hours. Each treatment, including the fungicide and the control (inoculated leaves without treatment), was replicated three times. After ten days of incubation, the percentage of affected leaf area was assessed to determine treatment efficacy.

Field Assessment of Organic and Inorganic Compounds on *Conocarpus* Seedlings. Field evaluation was conducted using one-year-old *Conocarpus* seedlings planted at the field research station of the College of Agricultural Engineering Sciences. Artificial infection was induced by spraying the seedlings with a fungal spore suspension at a concentration of 10^6 spores/mL.

Seven days after inoculation, the seedlings were treated with neem oil, sodium bicarbonate, and magnesium sulfate at concentrations of 0.2% and 0.4%. The fungicide Coastine 38WG (pyraclostrobin 12.8% + boscalide 25.2%) was applied as a comparative control, while untreated inoculated seedlings served as a positive control.

Experimental Design and Data Collection. The experiment was arranged in a randomized complete block design (RCBD) with five replicates per treatment. Following treatment application, the seedlings were maintained under standard agronomic practices, including regular irrigation and fertilization, for six months. Disease severity was assessed periodically, and growth parameters—including plant height and the average number of branches per seedling—were recorded.

Data Analysis. Statistical analysis was performed using SAS (version 9.4). Data were analyzed by ANOVA, and means were compared at $P \leq 0.05$. Percentage data were arcsine-transformed before analysis.

RESULTS AND DISCUSSION

Isolation and Identification of Fungi. Isolation from *Conocarpus* leaves exhibiting leaf spot symptoms revealed characteristic dark brown lesions on leaves and stems, accompanied by leaf chlorosis. Fungal isolates grown on PDA produced dark olive-colored colonies. Microscopic examination showed conidia that were both longitudinally and transversely septate and borne in chains on conidiophores, which is consistent with the morphological characteristics of *A. alternata* (Ellis, 1971) (Figure 2).

Pathogenicity of Fungal Isolates on *Conocarpus* Leaves Under Laboratory Conditions. The pathogenicity test results (Table 1) demonstrated that both fungal isolates exhibited high pathogenicity on *Conocarpus* leaves under laboratory conditions, with significant differences observed between the isolates. Isolate Alt.1IQ caused the highest level of infection, with an affected leaf area of 76.06% and an average lesion area of 8.70 cm^2. In contrast, isolate Alt.2IQ resulted in an affected leaf area of 68.13% and an average lesion area of 6.92 cm^2.

The observed variation in pathogenicity between the two isolates may be attributed to inherent genetic differences that influence virulence factors, enzymatic activity, and the ability to degrade host cell walls. These findings are consistent with previous reports documenting the aggressive nature of *A. alternata* infections on *Conocarpus* spp., are know the genus Alternaria have been identified as causing disease in Conocarpus (Ahmed et al., 2025).

Molecular Diagnosis of Pathogenic Fungi. Molecular identification of the fungal isolates was performed

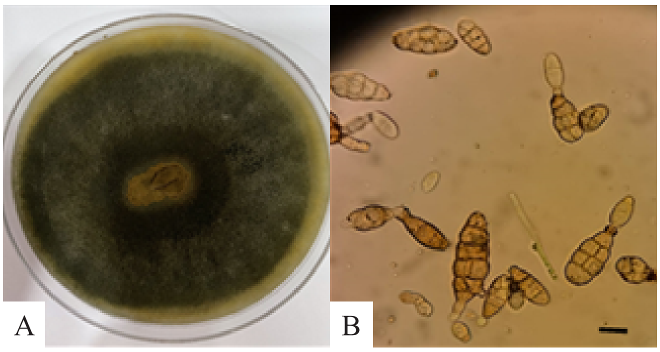

Figure 2. Morphological characteristics of *Alternaria alternata*. A. Colony growth of *A. alternata* on PDA medium; B. Conidia of *A. alternata* observed under a light microscope. Scale bars = 10 µm.

Table 1. Infestation rate and percentage of leaf area affected

The isolates	Leaf infection area (cm^2)*	Percentage of damaged leaf area (%)*
Alt.1IQ	8.70	76.06
Alt.2IQ	6.92	68.13
Control	0.00	0.00
L.S.D 0.05	0.70	4.38

*Each value represents the mean of six replicates. LSD (0.05) indicates the least significant difference at the 5% probability level.

using the universal primers ITS1 and ITS4. Agarose gel electrophoresis revealed clear amplification bands of approximately 527 bp and 542 bp for isolates Alt.1IQ and Alt.2IQ, respectively. Sequence analysis confirmed that both isolates belonged to *A. alternata*, showing identity values ranging from 98.05% to 100% when compared with reference sequences available in the NCBI GenBank database.

The nucleotide sequences of isolates Alt.1IQ and Alt.2IQ were deposited in GenBank under accession numbers OM994386 and OM994387, respectively (Table 2). BLAST analysis revealed that isolate Alt.1IQ (OM994386) shared 98.83% similarity with isolate MKL21463, previously isolated from citrus fruits, while isolate Alt.2IQ (OM994387) showed 99.61% similarity with isolate MW338656, isolated from apricot fruits (Figure 3). These results confirm the close genetic relationship between the Iraqi isolates and globally reported *A. alternata* strains.

Inhibiton of Fungal Pathogens by Specific Organic and Inorganic Compounds. The in vitro antifungal assay results (Table 3) demonstrated that all tested compounds significantly inhibited mycelial growth of *A. alternata* using the poisoned food technique. Magnesium sulfate and sodium bicarbonate exhibited the highest inhibitory effects at a concentration of 0.4%, with inhibition rates of 57.63% and 55.62%, respectively. Neem oil at the same concentration also showed strong antifungal activity, achieving a mycelial growth inhibition rate of 50.80%. In contrast, no inhibition was observed in the control treatment.

Variations in antifungal efficacy among the tested compounds and concentrations may be attributed to differences in fungal metabolic activity and enzyme production, which affect susceptibility to inhibitory substances (Ahmed et al., 2025).

The antifungal activity of sodium bicarbonate has been widely documented. Türkkan (2013) reported that sodium bicarbonate at 2% inhibited *Fusarium oxysporum* f.sp. *cepae*, by 54.2%. Similarly, Türkkan & Erper (2014) observed comparable inhibition levels against the same pathogen. Indra et al. (2019) demonstrated that increasing concentrations of sodium bicarbonate progressively suppressed the growth of *Macrophomina phaseolina* and *Fusarium* sp., while Piermann et al. (2022) reported complete inhibition of *Botrytis cinerea* at 10 mg/L. García et al. (2023)

Table 2. Genetic similarity among ITS regions of *Alternaria alternata* isolates from Iraq

No.	Common name	Botanical name	Accession number	Genetic similarity (%)		Reference
				Alt.1IQ	Alt.2IQ	
1	Buttonwood	*Conocarpus erectus*	OM994386.1	100	99.81	Current study
2	Buttonwood	*Conocarpus erectus*	OM994387.1	99.81	100	
3	Barley	*Hordeum vulgare*	MT874979.1	99.17	99.17	
4	Apricot tree	*Prunus armeniaca*	MK070043.1	99.79	99.79	
5	Wheat	*Triticum aestivum*	KX350053.1	98.75	98.74	
6	Jujube	*Ziziphus jujube*	OM976648.1	99.61	99.61	
7	Grapefruit	*Citrus paradisi*	OM562211.1	99.59	99.59	
8	Orange	*Citrus × sinensis*	MK121464.1	98.10	98.10	
9	Date palm	*Phoenix dactylifera*	OK235483.1	99.80	99.80	
10	Apricot	*Prunus armeniaca*	MW338656.1	99.61	99.61	https://blast.ncbi nlm.nih.gov/Blast.cgi
11	Orange	*Citrus × sinensis*	OM751881.1	99.61	99.61	
12	Apple	*Malus domestica*	OK073893.1	99.61	99.61	
13	Tomato	*Solanum lycopersicum*	KP864145.1	99.61	99.61	
14	Tomato	*Solanum lycopersicum*	MZ266750.1	99.80	99.80	
15	Oleander	*Nerium oleander*	LC621226.1	N.S.	N.S.	
16	Oleander	*Nerium oleander*	MT740389.1	98.05	98.05	
17	Orange	*Citrus × sinensis*	MK121463.1	98.83	98.83	
18	Orange	*Citrus × sinensis*	OM751880.1	99.61	99.61	
19	Fig	*Ficus carica*	MK070016.1	99.83	99.38	

Oleander (*Nerium oleander*); N.S.= No significant.

further confirmed concentration-dependent inhibition of *Colletotrichum* sp., reaching 100% inhibition at 1%.

Neem oil has also shown broad-spectrum antifungal activity. Duong et al. (2014) reported complete inhibition of *Rhizoctonia solani* and *Sclerotium rolfsii*, while Rawat et al. (2018) demonstrated significant suppression of several fungal pathogens, including *A. alternata.* Rodrigues et al. (2019) further confirmed

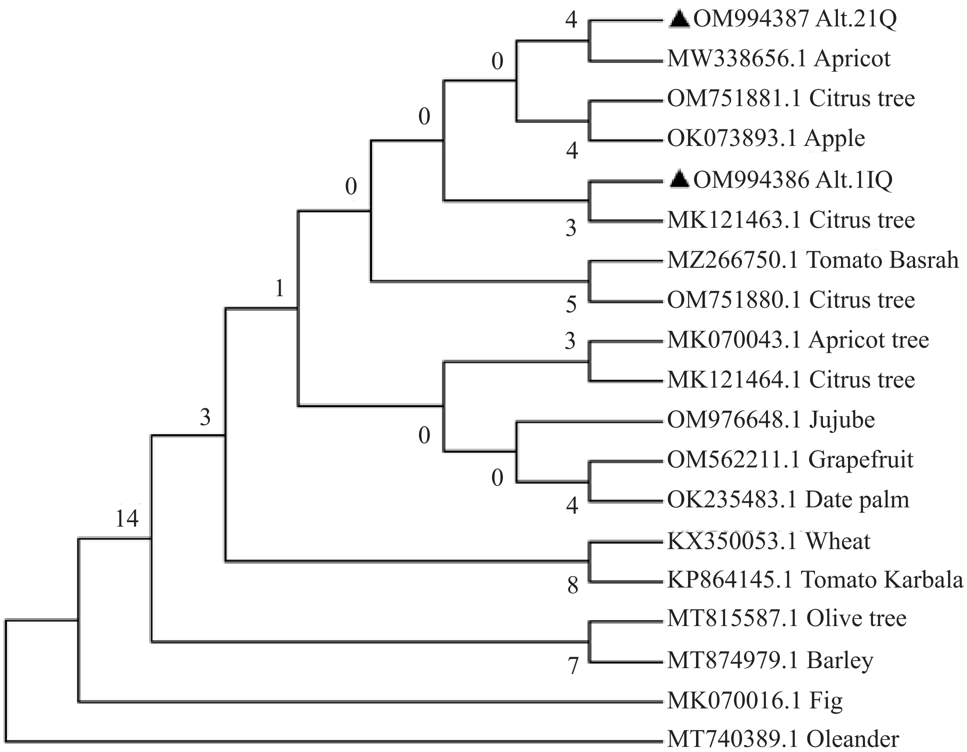

Figure 3. Molecular phylogenetic analysis using the maximum likelihood method based on ITS region sequences of 19 *Alternaria alternata* isolates from Iraq. The bootstrap consensus tree was inferred from 1000 replicates.

Table 3. Effectiveness of sodium bicarbonate, magnesium sulfate, and neem oil in inhibiting the growth of pathogenic fungi

Treatment	Concentration (%)	Growth rate (cm)		Mean growth (cm)	Inhibition rate %		Inhibition means %
		Alt.1IQ	Alt.2IQ		Alt.1IQ	Alt.2IQ	
Neem oil	0.1	5.37	4.38	4.88	40.30	51.27	45.79
Neem oil	0.2	5.08	4.27	4.68	43.47	52.53	48.00
Neem oil	0.4	4.93	3.92	4.43	45.17	56.43	50.80
Sodium bicarbonate	0.1	6.42	5.48	5.95	28.67	39.00	33.84
Sodium bicarbonate	0.2	6.05	5.40	5.73	32.73	39.93	36.33
Sodium bicarbonate	0.4	4.07	3.92	3.99	54.80	56.43	55.62
Magnesium sulfate	0.1	5.62	4.58	5.10	37.53	49.07	43.30
Magnesium sulfate	0.2	4.92	4.47	4.69	45.37	50.37	47.87
Magnesium sulfate	0.4	4.03	3.58	3.81	55.13	60.13	57.63
Control	-	8.50	8.50	8.50	0	0	0
LSD (0.05)	-	0.44	0.31	4.92		3.48	

*Values represent the mean of six replicates. LSD (0.05) indicates the least significant difference at the 5% probability level.

that neem oil at low concentrations (0.3%) effectively inhibited *Aspergillus carbonarius*.

Efficacy of Botanical and Chemical Compounds Against Leaf Spot Disease. The leaf assay results (Table 4) showed that neem oil, sodium bicarbonate, magnesium sulfate, and the fungicide Coastine 38WG effectively prevented the development of leaf spot symptoms on *Conocarpus* leaves. At concentrations of 0.2% and 0.4%, all tested compounds completely suppressed disease development, resulting in 0% affected leaf area. In contrast, the untreated control exhibited a mean affected leaf area of 31.96%.

The effectiveness of these treatments is supported by previous studies. Neem oil acts as a potent antifungal agent due to its bioactive constituents, which interfere with fungal growth and reproduction (Rodrigues et al., 2019). Sodium bicarbonate suppresses fungal pathogens by increasing surface pH, thereby creating unfavorable conditions for fungal development (Türkkan & Erper, 2014). Magnesium sulfate may influence fungal growth through its role in cellular metabolism and enzyme regulation (Kolaei et al., 2012).

Management of Leaf Spot Disease in *Conocarpus* Seedlings Using Organic and Inorganic Compounds. Field evaluation results (Table 5) that all tested treatments significantly reduced leaf spot disease severity compared to the pathogenic fungus-only and untreated control treatments. Neem oil reduced disease severity to 16.6% and 8.3% at concentrations of 0.2% and 0.4%, respectively. Sodium bicarbonate resulted in disease severity values of 25% and 8.3% at the same

Table 4. Effectiveness of sodium bicarbonate, magnesium sulfate, neem oil, and Coastine 38WG in inhibiting fungal growth on *Conocarpus* leaves

Treatment	Concentration (%)	Rate of lesion expansion on leaves (cm^2)*	Percentage of leaf area affected (%)*
Neem oil	0.2	0.00	0.00
Neem oil	0.4	0.00	0.00
Sodium bicarbonate	0.2	0.00	0.00
Sodium bicarbonate	0.4	0.00	0.00
Magnesium sulfate	0.2	0.00	0.00
Magnesium sulfate	0.4	0.00	0.00
Coastine 38WG	-	0.00	0.00
Control	-	2.61	31.96
LSD (0.05)	-	0.32	3.93

*Values represent the mean of three replicates. LSD (0.05) indicates the least significant difference at the 5% probability level.

Table 5. Efficacy of neem oil, sodium bicarbonate, magnesium sulfate, and Coastine 38WG in controlling leaf spot disease for *Conocarpus* Seedlings under nursery

Treatment	Concentration (%)	Disease severity*	Number of branches*	Plant height (m)*
Neem oil	0.2	16.60	46.67	2.00
Neem oil	0.4	8.30	54.67	2.07
Sodium bicarbonate	0.2	25.00	45.33	1.73
Sodium bicarbonate	0.4	8.30	50.00	2.15
Magnesium sulfate	0.2	25.00	42.00	1.90
Magnesium sulfate	0.4	24.33	47.33	2.22
Pathogenic fungus	-	91.60	23.00	1.27
Coastine 38WG	-	33.30	39.67	1.63
Control	-	00.00	36.33	1.53
LSD (0.05)	-	0.94	5.44	0.20

*Values represent the mean of five replicates. LSD (0.05) indicates the least significant difference at the 5% probability level.

concentrations. Magnesium sulfate showed moderate effectiveness, with disease severity values of 25% and 24.33%. The fungicide Coastine 38WG completely suppressed disease symptoms, while seedlings inoculated with the pathogen alone exhibited a disease severity of 91.6%. These findings align with previous studies that have utilized organic and inorganic salts to control plant pathogens and induce resistance in plants (Rodrigues et al., 2019).

All treatments also significantly enhanced vegetative growth parameters. Neem oil at 0.4% resulted in the highest number of branches per plant (54.67), followed by sodium bicarbonate at 0.4% (50.00). Magnesium sulfate at 0.4%, neem oil at 0.2%, and sodium bicarbonate at 0.2% produced 47.33, 46.67, and 45.33 branches per plant, respectively, compared to only 23.00 branches in the pathogen-only treatment.

Plant height measurements showed no significant differences among neem oil, sodium bicarbonate, and magnesium sulfate at 0.4%, with values of 2.07, 2.15, and 2.22 m, respectively. These values were significantly higher than those observed in pathogen-inoculated seedlings, which reached only 1.27 m.

The effectiveness of sodium bicarbonate has been attributed to its alkaline properties, which disrupt fungal cell membrane stability and enzyme activity (Türkkan et al., 2018). Neem oil contains numerous secondary metabolites, including azadirachtin and related terpenoids, which suppress fungal growth and protect chlorophyll integrity, thereby enhancing photosynthetic efficiency and plant development (Rodrigues et al., 2019).

Magnesium sulfate contributes to disease suppression by improving plant nutritional status and activating physiological defense mechanisms, as magnesium plays a crucial role in chlorophyll synthesis and enzyme activation (Kolaei et al., 2012; Rabea et al., 2023).

CONCLUSION

This study confirmed that *Alternaria alternata*, the causal agent of leaf spot disease, is an emerging and highly destructive pathogen of *Conocarpus* plants, with a rapidly increasing incidence. The pathogen was accurately identified through combined morphological and molecular characterization, providing definitive evidence of its role in disease development. The results demonstrated that neem oil, sodium bicarbonate, and magnesium sulfate have strong potential as eco-friendly alternatives to conventional chemical fungicides for the management of *A. alternata*. In vitro assays revealed significant inhibition of fungal growth by all tested compounds. Moreover, field evaluations on *Conocarpus* showed a substantial reduction in disease severity following treatment with these substances, confirming their practical applicability under field conditions. Overall, the use of organic and inorganic compounds represents a promising and sustainable strategy for managing leaf spot disease in Conocarpus, particularly in environments where reliance on chemical fungicides poses environmental and health concerns.

ACKNOWLEDGMENTS

The authors sincerely thank their colleagues in the Department of Plant Diseases, College of Agricultural Engineering Sciences, University of Baghdad, Iraq, for their valuable cooperation and technical support throughout the course of this study.

FUNDING

This research received no external funding.

AUTHORS' CONTRIBUTIONS

EKAK and HAA conceived and designed the research. EKAK, HAA, and TAK conducted the experiments and collection the data. TAK performed the statistical analyses. EKAK drafted the manuscript, HAA prepared the figures and tables. All authors contributed to data interpretation, critically revised the manuscript, and approved the final version.

COMPETING INTEREST

The authors declare that they have no competing interests. The research was conducted independently, without any financial or personal relationships that could have influenced the study's design, execution, or interpretation of the results.

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