Research Article

Bicarbonate Salts and Some Vegetable Oils Use for Control of Swiss Chard Leaf Spot Caused by the Fungus *Cercospora beticola*

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Abstract | This study was conducted to evaluate the effectiveness of sodium bicarbonate and certain plant-derived oils (clove and sesame) in managing Cercospora leaf spot disease on Swiss chard. Survey results indicated the presence of the disease in all examined locations, with Thi Qar Governorate showing the highest infection rate at 40.155%, compared to 24.365% in Babylon Governorate. Variation in disease severity was also noted across different regions within the same province. Laboratory analysis confirmed that *Cercospora beticola* was the primary pathogen, with symptoms characterized by small, circular, brown lesions with distinct reddish-purple margins. Microscopically, fungal colonies appeared gray and velvety, and the conidia were elongated, transparent, straight or slightly curved, with an inverted club-like shape, rounded bases, tapered ends, and contained 5–10 transverse septa. Antifungal efficacy tests demonstrated that all treatments reduced fungal growth, with sodium bicarbonate proving the most effective. The inhibitory effect increased with higher concentrations, with 1000 ppm achieving the highest inhibition rate of 95.12%. All treatments significantly decreased disease severity, with sodium bicarbonate reducing it to 23.84% compared to 42.92% in the untreated control. Clove and sesame oil treatments reduced severity to 28.34% and 32.41%, respectively, while the chemical fungicide Folicur recorded the lowest severity at 12.89%. Disease incidence followed a similar trend, where sodium bicarbonate achieved a 44.45% reduction, clove oil 33.97%, sesame oil 24.48%, and the chemical fungicide 69.93%. Additionally, all treatments enhanced total chlorophyll content in the leaves, with sodium bicarbonate reaching 9.18 mg, clove oil 8.57 mg, sesame oil 7.36 mg, compared to 5.34 mg in the control, while the Folicur treatment recorded the highest value at 11.38 mg. These findings highlight the potential of sodium bicarbonate and natural plant oils as eco-friendly alternatives to chemical fungicides in controlling Cercospora leaf spot in Swiss chard.

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Introduction

Swiss chard is a leafy vegetable widely cultivated throughout the world due to its numerous benefits. Swiss chard (*Beta vulgaris*) belongs to the Chenopodiaceae family (Matloub, 1989) and is rich in minerals, especially Fe, K, and Mg, as well as vitamins A, B, and C (Al-Qabbani, 2006). It is used to treat

stomach ulcers, as it strengthens the gastric mucosa and protects against cancer (Aguirre and May, 2008). Swiss chard oil, extracted from its seeds, is also effective against tapeworms and roundworms (Al-Kateb, 1988).

Swiss chard is affected by several pathogens that reduce its market value. These include Fusarium wilt, caused by *Fusarium oxysporum* fungus, powdery mildew, caused by *Erysiphe betae* fungus, and root rot, caused by *Phoma betae* fungus. Cercospora leaf spot, caused by *Cercospora spp.* fungus, it is one of the most important diseases causing poor quality leaves produced by this crop (Zhang *et al.*, 2014). The efforts of scientists and researchers from most countries of the world have focused on finding or developing alternatives to chemical pesticides that are safe, non-toxic to humans, animals, and the environment, and are rapidly decomposable. Among these alternatives are bicarbonate salts and vegetable oils (Lahhob *et al.*, 2025; Tarmooz *et al.*, 2025). Due to the frequent consumption of Swiss chard by many Iraqi households, there is a growing concern over potential exposure to mycotoxins produced by fungi responsible for leaf spot diseases on this crop. Given the crop's economic and nutritional significance, and the scarcity of comprehensive studies addressing this issue, the need for this research became evident.

This study aims to investigate the effectiveness of sodium bicarbonate and selected plant-based oils (clove and sesame) in suppressing the growth of leaf spot-causing fungi on Swiss chard. Additionally, it evaluates the performance of these natural alternatives in comparison with the chemical fungicide Folicur and assesses their impact on enhancing the growth of infected plants (Hasan *et al.*, 2025).

Materials and Methods

Field survey

A field survey was conducted on a number of fields planted with Swiss chard (Table 1), in the provinces of Thi Qar (Al-Gharraf and Al-Fajr) and Babylon (Al-Shomali and Al-Musayyab). Two fields were randomly selected from each region. The survey was conducted by dividing the field into four sections. Plants were then randomly taken from each section. Plants were divided into categories within each plant sample according to the pathology guide, developed to describe symptoms in Swiss chard plants (Figure 1).

Then the percentage of disease severity was calculated according to the Mckinney equation (1923) as follows:

$$Severity\ (\%) = \frac{\text{Total (plants examined number for each grade} \times \text{its grade)}}{\text{Total number of plants examined} \times \text{highest score}} \times 100$$

Degree	Details
0	Healthy plant
1	1-6 spots/leaf
2	7-12 spots/leaf
3	13-19 spots/leaf
4	20 spots or more/leaf

Table 1: *Places and dates of sampling of Swiss chard plants infected with Cercospora leaf spot.*

Governorate	Regions	Sampling date	Cultivated variety
Thi Qar	Al-Gharraf	20/12/2024	Lucullus
	Al-Fajr	28/12/2024	Local
Babylon	Al-Shomali	5/1/2025	White silver
	Al-Musayyab	10/1/2025	Local

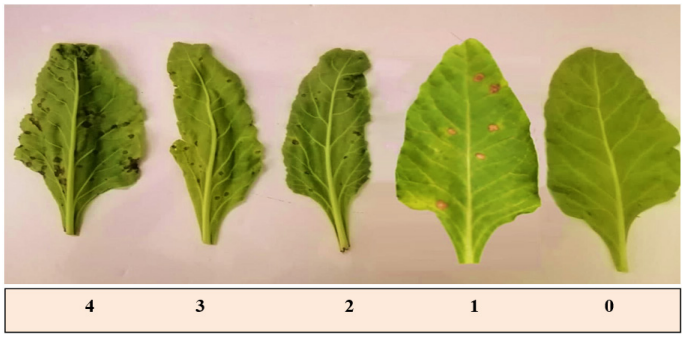

Figure 1: *Pathological guide to measure the severity of infection with pathogenic fungi.*

Isolation and identification of the pathogenic fungus

Symptomatic Swiss chard leaves were collected from surveyed field sites and thoroughly rinsed under running water to eliminate surface debris. The samples were then air-dried and cut into segments measuring approximately 0.5–1 cm. Surface sterilization was carried out using a 1% sodium hypochlorite (free chlorine) solution for three minutes, followed by rinsing with sterile distilled water to remove any residual disinfectant. The segments were then dried using Whatman No. 1 filter paper. Subsequently, 3–4 pieces were placed on 9 cm Petri dishes containing sterilized potato dextrose agar (PDA) medium. The plates were incubated at 25°C to promote fungal growth. Once mycelial growth was observed, purification was achieved by transferring the hyphal tips onto fresh sterile PDA plates. These were incubated under the

same conditions for seven days. Fungal isolates were then identified based on morphological characteristics using standard taxonomic keys (Ellis, 1971; Behrooze *et al.*, 2017).

Purification and preservation of the fungus that causes Swiss chard leaf spot

Fresh growths were taken from the edges of *C. beticola* colony, these were grown in a 9 cm Petri dish containing sterile PDA culture, incubated at 25°C for 7 days, after ensuring pure growth, a portion of the colony was transferred to sterile plastic tubes containing culture medium at an angle, incubated at the same temperature and for the same duration and refrigerated until use.

Pathogenicity testing of the fungus causing swiss chard leaf spot

The pathogenic potential of *Cercospora beticola* was evaluated using plastic pots filled with a sterilized soil and peat moss mixture in a 1:2 ratio. Sterilization was performed using commercially available formalin. Locally sourced Swiss chard seeds were sown at a density of eight seeds per pot. Following germination, seedlings were thinned to retain a single healthy plant per pot. Once the plants reached 50 days of age, the aerial parts were inoculated with a *C. beticola* spore suspension prepared at a concentration of 10^4 spores/mL. A sterile 500 mL hand sprayer was used for application, and the spore concentration was calibrated using a hemocytometer. To maintain optimal humidity for infection, the inoculated plants were enclosed in plastic bags for 48 hours. Control plants were treated with sterile distilled water only. After a period of three weeks, disease severity was assessed using the previously described scale. The experiment was performed in triplicate to ensure reproducibility.

Testing the effect of sodium bicarbonate and some tested vegetable oils on the growth of C. beticola fungi on PDA culture media

The experiment was carried out in the Plant Pathology Laboratory of the Department of Plant Production Technologies at the Shatra Technical Institute, Southern Technical University. The vegetable oils and sodium bicarbonate used in the subsequent tests were sourced from local markets. Treatment solutions were prepared at concentrations of 100, 500, and 1000 ppm. To prepare these, 0.5 grams of each treatment material—vegetable oil (clove or sesame), sodium bicarbonate, and the chemical fungicide Folicur—were each dissolved separately in 50 mL of distilled water. From these stock solutions, 1, 5, and 10 mL were taken and added to 99, 95, and 90 mL of sterilized potato dextrose agar (PDA) medium, respectively, cooled to approximately 45°C. The amended media were then poured into Petri dishes (9 cm in diameter), with three replicates prepared for each treatment and concentration. Control plates were prepared by adding only sterile distilled water to the PDA medium. Once solidified, each plate was inoculated at the center with a 0.5 cm disc cut from a seven-day-old fungal culture grown on PDA. All plates were incubated at 25°C for seven days. The diameter of the fungal colonies was recorded once the fungal growth in the control plates reached the edge of the dish. The percentage of fungal growth inhibition was calculated using the following formula:

$$Inhibition\ (\%) = \frac{\text{Fungi growth rate in control treatment} - \text{Fungi growth rate}}{\text{Fungi growth rate in control treatment}} \times 100$$

The dishes were distributed according to a completely randomized design (C.R.D.). The results were analyzed and compared statistically using the least significant difference (LSD) test at a probability level of 0.01 (Al-Rawi and Khalaf Allah, 1980).

Evaluation of the effectiveness of sodium bicarbonate and some tested vegetable oils in controlling Swiss chard leaf spot in the field

The field experiment was conducted during the 2024–2025 winter growing season in one of the agricultural fields affiliated with the Department of Plant Production Technology at the Shatra Technical Institute, Southern Technical University. Following plowing and land preparation, the field was leveled and divided into plots of 2 m^2 each. White Silver Swiss chard seeds, produced by the Dutch company Enza Zaden, were sown. Standard agronomic practices and crop management operations were carried out as required throughout the growing period. Four weeks after sowing, the plants were fertilized with urea every 14 days. Upon reaching 40 days of age, the plants were inoculated with a *Cercospora beticola* spore suspension. The suspension was prepared using a 21-day-old fungal colony grown on PDA medium. Sterile distilled water (10 mL) was added to the culture plate, and a sterile soft brush was used to gently dislodge the spores. The resulting suspension was transferred into a sterile test tube and further diluted with distilled

water. A haemocytometer was used to adjust the spore concentration to 10^4 spores/mL, following the method described by Fayyad (2021).

The spore suspension was applied using a sterile hand sprayer until the foliage was fully saturated. Ten days post-inoculation, treatments were applied: sodium bicarbonate at 4 g/L, and plant oils (clove and sesame) at 4 mL/L, using a 5-liter hand sprayer. The sprayer was thoroughly rinsed with sterile distilled water between treatments to prevent cross-contamination. Folicur, a systemic fungicide containing tebuconazole (a triazole compound) and manufactured by Bayer (Germany), was applied at 1 mL/L until the foliage was completely wetted. Control plots were sprayed with sterile distilled water only. The experimental design followed a randomized complete block design (RCBD) with three replicates per treatment. Treatment means were statistically compared using the Least Significant Difference (LSD) test at the 0.05 probability level (Hasan *et al.*, 2022).

The experiment included the following treatments:

1. Control treatment.
2. Sodium bicarbonate treatment.
3. Clove oil treatment.
4. Sesame oil treatment.
5. Folicur chemical pesticide treatment.

Ten days after treatment application, disease severity on Swiss chard plants was assessed using the disease index as described previously. The percentage of disease reduction was subsequently determined using the following formula:

$$\text{Reduction rate (\%)} = \frac{\text{Severity by comparison} - \text{Severity by treatment}}{\text{Severity by comparison}} \times 100$$

(Maharjan *et al.*, 2015).

Estimation of total leaf chlorophyll content (mg per 100 g fresh weight)

Total chlorophyll content in Swiss chard leaves was estimated following the method. Leaf samples were collected from each treatment group and weighed using a precision analytical balance. The fresh leaves were ground in a ceramic mortar with the addition of 10 mL of 80% acetone to facilitate pigment extraction. The homogenate was filtered through standard filter paper to obtain the chlorophyll-containing solution. The filtrate was then transferred into centrifuge tubes and centrifuged at 6000 rpm for 10 minutes. The resulting supernatant was collected and analyzed using a spectrophotometer to measure absorbance at wavelengths of 663 nm for chlorophyll and 645 nm for chlorophyll b. The total chlorophyll content (mg/L) was calculated using the appropriate equation.

Total chlorophyll (mg/L) = 20.2 × O.D (645) + 8.02 × O.D (663)

O.D represents the absorbance reading of the device. To convert the amount of chlorophyll from (mg/L) to (mg/100 gm) sample weight, the following equation was applied:

$$1\ mg.100\ gm = \frac{mg/L}{1000\ ml} \times \frac{100}{Sample\ weight\ measured\ in\ grams}$$

Table 2: *The severity of infection with Swiss chard leaf spot disease in different areas of Thi Qar and Babylon governorates.*

Governorate	Regions	Severity (%)	Mean
Thi Qar	Al-Gharraf	43.52	40.155
	Al-Fajr	36.79	
Babylon	Al-Shomali	21.43	24.365
	Al-Musayyab	27.30	

$L.S.D_{0.05}$ =2.19

Results and Discussion

Field survey of the severity of infection with cercospora leaf spot disease in swiss chard

Table 2 presents the results of the field survey on the prevalence of *Cercospora* leaf spot disease on Swiss chard during the 2024–2025 growing season. The highest overall infection severity was observed in Thi Qar Governorate, reaching 40.155%, whereas Babylon Governorate exhibited a lower severity of 24.365%. Within the same governorate, infection levels varied across different areas. In Thi Qar, the Al-Gharraf region showed the highest severity at 43.52%, compared to 36.79% in the Al-Fajr region, which may be attributed to differences in humidity. Similarly, variations were observed across the regions of Babylon Governorate, with Al-Shomali exhibiting the highest severity at 27.30%, compared to 21.43% in Al-Musayyab.

Symptoms of infection appear as small, round, brown

to reddish-purple spots with defined edges. These spots dry up, leaving holes in their place. The infection appears on the lower leaves and then spreads to the upper leaves. Infection also appears on the leaf petioles, agreed with Crous and Braun (2003), Agrios (2005), and Franc (2010), who reported that the severity of Swiss chard leaf spot increases with increased humidity, temperature, and the use of pathogen-bearing seeds (Hasan *et al*., 2024) and (Abdullah *et al*., 2025).

Isolation and identification of the fungus causing Swiss chard leaf spot

The fungus *Cercospora beticola* was isolated and identified from chard leaves showing symptoms. The fungus formed small, round, brown spots with well-defined edges that tended to be reddish-purple. Colonies had a velvety gray appearance. Under a microscope (Figure 2), long, transparent, straight or slightly curved conidia appeared, with an inverted club-shaped shape, a rounded base, and a tapered apex. They were divided into 5-10 transverse septa. These characteristics were consistent with Ellis (1971) and Behrooze *et al*. (2017).

Figure 2: *Colonies and spores of the fungus C. beticola. A. Spores of the fungus C. beticola under a microscope at 40x magnification. B. Colony of the fungus C. beticola on PDA culture media.*

Pathogenicity of cercospora beticola fungus

As shown in Figure 3, *C. beticola* fungus isolated from Swiss chard leaves possesses a high pathogenicity, with an infection severity of 41.11%. The fungus caused leaf spot symptoms to appear 8-12 days after inoculation. Symptoms appeared on the leaves as small, round, brown to reddish-purple spots with defined edges, ranging in diameter from 2-7 mm. These spots are covered with gray to black growth as spores form. These spots then dry, leaving holes in their place. The infection initially appeared on the lower leaves, then it spread to the upper leaves. These symptoms were similar to those of the previously isolated infected plants. Upon re-isolation, the same fungus was obtained from the inoculated plants, which supports its role as the pathogen. These symptoms were agreed with Fajola (1978); Daub and Ehrenshaft (2000); Goudet *et al*. (2000). The pathogenic fungus also enters the plant through the stomata. It spreads through the intercellular spaces and secretes non-specific toxins, namely beticolin and cercosporin (Steinkamp *et al*., 1979).

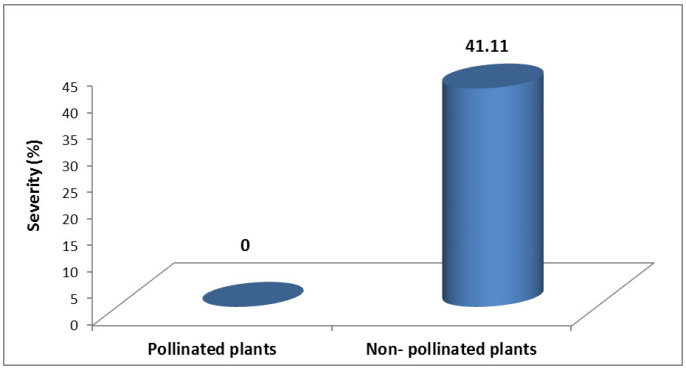

Figure 3: *Testing the pathogenicity of C. beticola fungus on Swiss chard.*

Table 3: *Effect of sodium bicarbonate and some tested vegetable oils on the growth of C. beticola on PDA media.*

Treatments	Concentrations (ppm)		
	100	500	1000
Control	0.0	0.0	0.0
Sodium bicarbonate	77.59	84.73	9512
Clove oil	65.66	76.33	86.87
Sesame oil	46.21	52.21	63.45
Folicur chemical pesticide	100	100	100
$L.S.D_{0.01}$	3.936	5.356	3.329

The effect of sodium bicarbonate and some tested vegetable oils on the growth of C. beticola on PDA culture media

Table 3 indicates that all treatments used in the study inhibited the growth of the pathogenic fungus. However, the inhibition rate varied from one treatment to another and from one concentration to another. The inhibition rate increased with increasing concentration, and the sodium bicarbonate treatment had the greatest effect. The inhibition rate reached 77.59, 84.73 and 95.12% at concentrations of 100, 500, and 1000 ppm, respectively. Then, the clove oil treatment showed inhibition rates of 65.66, 76.33 and 86.87%. Then, the sesame oil treatment showed inhibition rates of 46.21, 52.21 and 63.45% at the same three concentrations, respectively, compared to the control treatment, which showed an inhibition rate of 0%. While the inhibition rate in the standard

treatment of the chemical pesticide Folicur 100% for all concentrations in the same order (Hasan *et al*., 2023).

The inhibitory effect of sodium bicarbonate salts is attributed to the change in osmotic pressure and pH. These salt solutions act by contact and inhibit the growth of fungal hyphae. The inhibitory effect of bicarbonate salts may be due to the decrease in turgor pressure, which leads to the collapse and shrinkage of fungal hyphae (Türkkan *et al*., 2017).

Sodium and potassium bicarbonates affect the balance of sodium and potassium ions in pathogenic fungal cells, leading to cell wall collapse. The mechanism of the effect of vegetable oils on conidia and hyphae cells may be due to disruption of the permeability of the membranes of fungal hyphae and conidia cells, leading to loss of water content and leakage of cell contents, resulting in a shrunken appearance (Wurms *et al*., 2018).

Some of these compounds may interfere with many of the vital functions of pathogenic fungi, because they affect certain enzymes essential for fungal vitality, they inhibit their function and halt fungal growth. Numerous studies have indicated that clove plant tissues contain many phenolic compounds, these contain a high percentage of euginol, comprising 70-90% of the plant's composition. Euginol possesses a high inhibitory capacity against many fungal pathogens and causes thinning of funga l cell walls, disruption of the plasma membrane, and distortion of mitochondria (Karim, 2000; Alma *et al*., 2007).

The effect of sodium bicarbonate and some tested vegetable oils on controlling Swiss chard leaf spot in the field

Table 4 shows that all treatments used in the experiment significantly reduced the percentage of Swiss chard leaf spot infection severity after ten days of treatment. The sodium bicarbonate treatment significantly reduced the percentage of infection severity, decreasing from 42.92% in the control treatment to 23.84% in the sodium bicarbonate treatment. Meanwhile, the percentage of infection severity in the clove oil treatment reached 28.34%, followed by the sesame oil treatment with a percentage of 32.41%. The percentage of infection severity in the standard Folicur treatment reached 12.89%.

As noted, that all treatments were effective in reducing the disease. The sodium bicarbonate treatment yielded the highest disease reduction rate, reaching 44.45%. The clove oil treatment followed, with a disease reduction rate of 33.97%. The sesame oil treatment then yielded a disease reduction rate of 24.48%. The disease reduction rate in the standard chemical pesticide treatment was 69.93%. Jaber *et al*. (2025)

Table 4: *The effect of sodium bicarbonate and some tested vegetable oils in controlling Swiss chard leaf spot disease in the field.*

Treatments	Severity (%)	Treatments efficiency (%)
Control	42.92	-
Sodium bicarbonate	23.84	44.45
Clove oil	28.34	33.97
Sesame oil	32.41	24.48
Folicur chemical pesticide	12.89	69.93
$L.S.D_{0.01}$	1.92	1.78

There are several potential mechanisms involved in the effect of bicarbonate salts on pathogenic fungi. These include an increase in leaf surface pH and the breakdown of pathogenic fungal cell walls. This can be due to an imbalance in sodium ions or the desiccation of pathogenic fungal spores. The use of sodium bicarbonate salts can reduce the potential for the development of resistance to pathogenic fungi compared to chemical pesticides (Zir and Zitter, 1992). The effect may be due to bicarbonate's ability to increase the concentration of the enzymes B-1,3-glucanase and chitinase. These enzymes break down B-1,3-glucan and chitin found in the cell wall of pathogenic fungi, leads to cell death (Abd-El-Kareem and Abd-El-Latif, 2012). The mechanism by which oils affect conidia may be due to disruption of the permeability of the membranes of hyphae and conidia. This leads to loss of water content and leakage of cell contents, resulting in a shrunken appearance, this was consistent with Ko *et al*. (2003) reported, which stated that conidia treated with oils fail to germinate and become shrunken and deformed. Alternatively, the antimicrobial activity of oils against microorganisms may be due to a number of chemical compounds, including Carvacrol and Thymol. Other studies have indicated that cloves contain numerous phenolic compounds in their tissues, including a high percentage of euginol, reaching 70-90%. This compound possesses a high inhibitory capacity against many fungal pathogens. It causes thinning of

cell walls, disruption of the plasma membrane, and distortion of mitochondria (Alma *et al*., 2007).

Table 5: *The effect of sodium bicarbonate and some tested vegetable oils on the chlorophyll content of chard.*

Treatments	Chlorophyll content (mg gm^{-1})
Control	5.34
Sodium bicarbonate	9.18
Clove oil	8.57
Sesame oil	7.36
Folicur chemical pesticide	11.38
L.S.D$_{0.01}$	0.760

The effect of sodium bicarbonate and some tested vegetable oils on the chlorophyll content of chard

Table 5 shows that all treatments applied in this study resulted in an increase in the total chlorophyll content of Swiss chard leaves. The highest chlorophyll content was observed in plants treated with sodium bicarbonate (9.18 mg/g), followed by clove oil (8.57 mg/g) and sesame oil (7.36 mg/g), compared to the control, which recorded 5.34 mg/g. The standard chemical fungicide Folicur exhibited the highest chlorophyll content overall at 11.38 mg/g Khadhum *et al*., (2025).

The elevated chlorophyll levels observed in the oil and sodium bicarbonate treatments can be attributed to the reduced severity of leaf spot disease, which limits the area of infected tissue compared to the control. Leaf spot diseases generally impair photosynthesis by causing tissue necrosis and reducing the green leaf area (Agrios, 2005). *Cercospora* fungi produce the non-specific mycotoxin cercosporin, which contributes to disease severity and further diminishes photosynthetic efficiency. These findings are in agreement with Groenwald (2013) and Bakhshi *et al*., (2018), who reported that *Cercospora* infection leads to leaf tissue damage, decreased photosynthesis, and consequently, reduced crop productivity and efficiency.

Conclusions

Cercospora beticola fungus is widespread on Swiss chard plants in Thi Qar and Babylon provinces. The use of sodium bicarbonate, clove oil, and sesame oil reduced the severity of leaf spot disease on Swiss chard plants, in addition to their high inhibitory activity against the pathogenic fungus. The effectiveness of the treatments used varied depending on the concentration and type of treatment. The use of sodium bicarbonate and some tested vegetable oils increased the amount of chlorophyll and improved growth parameters of Swiss chard plants inoculated with *C. beticola*.

Acknowledgments

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Novelty Statement

The study discusses the evaluation of the efficiency of sodium bicarbonate and some vegetable oils (clove and sesame) in combating Cercospora leaf spot disease on chard plants. The results indicated the spread of the disease on chard plants in all the areas covered by the survey, and the highest severity of the disease was recorded in Dhi Qar Governorate, reaching 40.155%, compared to 24.365% for Babylon Governorate. The results also showed a variation in the percentage of severity of the disease between the areas belonging to the same governorate.

Author's Contribution

Muthafar Khshan Khadhum and Hasanain Ali Jaber: study concept methodology, data analysis, manuscript preparation.
Saifuldeen Ahmed Hasan: data validation and final editing.
Raeed Mejbel Abdullah: technical support and consulting.
All authors approved the final version of the manuscript and are responsible for the submitted material.

Generative AI and AI-assisted technology statement

The artificial intelligence analysis of all sections of this manuscript showed that none of the text plagiarized and was with a score of zero.

Conflict of interest

The authors have declared no conflict of interest.

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