Journal of Plant Protection Research eISSN 1899-007X

ORIGINAL ARTICLE

Biocontrol of *Sphaerotheca fuliginea*, the causal agent of powdery mildew of cucumber by using aqueous extracts from five traditional Egyptian medicinal plants

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Vol. 63, No. 1: 39–49, 2023

DOI: 10.24425/jppr.2023.144500

Received: August 23, 2022
Accepted: October 14, 2022
Online publication: February 01, 2023

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Responsible Editor:
Jolanta Kowalska

Abstract

The goal of this study was to evaluate the effectiveness of aqueous extracts from five traditional Egyptian medicinal plants in preventing *Sphaerotheca fuliginea*'s powdery mildew disease, which affects cucumber plants. Aqueous extracts from each of the examined plants suppressed the pathogen's conidia germination *in vitro*. In trials using detached leaves and greenhouses, these extracts lessened the severity of the disease. Compared to other plant extracts, *Curcuma longa* rhizome extract showed the greatest potency against the pathogen. The aqueous extract of *Curcuma longa* showed the largest improvement in disease suppression compared to the control in the greenhouse experiment. The results showed that total phenol and associated defense enzyme levels (POD and PPO) were elevated by plant extracts from all studied plants. These findings might suggest that total phenol and associated defense enzymes strengthen the cucumber's resistance to the disease. The *C. longa* extract had more total phenol than the extracts from the other plants. The phenolic components in the *C. longa* rhizome extract were varied, and these variations were detected and quantified using high-performance liquid chromatography (HPLC). The content of curcumin (3220.8 $\mu g \cdot g^{-1}$ dry weight) was the highest. In comparison to the control, the foliar application of the *C. longa* extract considerably increased the cucumber fruit yield and its constituent parts. This is the first time, to my knowledge, that the *C. longa* rhizome extract has been utilized to improve cucumber plants' production and its constituent parts. The pathogen appeared as small colonies with fewer mycelia and immature conidia in the treated cucumber leaves with 20% of *C. longa* rhizome extract according to an examination by SEM. Overall, the results indicated that the extract of *C. longa* rhizome, was a promising, effective, and environmentally friendly management measure against powdery mildew disease of cucumbers, and thus could be used in the production of organically grown vegetables.

Keywords: cucumber, defense enzymes, fungal plant diseases, induced resistance, medicinal plants, phenolic compounds

Introduction

Powdery mildew caused by *Sphaerotheca fuliginea* (Schlect.) Pollachi is the most significant disease affecting cucumbers produced in fields and greenhouses in Egypt, and it must be controlled with high concentrations of fungicides. It is not advisable to use fungicides extensively due to environmental concerns and the emergence of infections that are resistant to them. As a result, alternative powdery mildew control measures must be devised to reduce fungicide input and reliance on these fungicides.

Because plant extracts are an effective source of bioactive substances such as phenols, flavonoids, quinines, tannins, alkaloids, saponins, and sterols, they may be utilized as an alternative to fungicides

to control phytopathogenic fungi (Burt 2004). These extracts become safer control agents since they are effective against fungal plant pathogens, biodegrade to harmless substances, and may be suitable for use in integrated pest management programs.

For thousands of years, Egypt's traditional medicinal herbs have been an integral part of its natural and cultural legacy. Ancient Egyptians were equally familiar with making medicines from plants and herbs such as tamarind, licorice, henna, bitter apple, and turmeric (Aboelsoud 2010). Over the past several years, there has been an increase in the quest for plant extracts with anti-ascomycete activity. These studies have shown that plant extracts are effective against *S. fuliginea*, the fungal pathogen that causes the powdery mildew disease that affects Cucurbitaceae (Al Surhanee 2013; Guginski-Piva *et al*. 2015; Abdu-Allaha and Abo-Elyousr 2017; El-Ghanam *et al*. 2018; Mostafa *et al*. 2021a)

There are several ways that plants defend themselves from diseases, such as phenolic complexes, which are secondary plant metabolites. These substances can be found in the root, rhizome, shoot, leaf, and flower of the plant (Houssien *et al*. 2010). They suggested that phenolic intermediates play a part in the active expression of resistance. Induced resistance is a relatively new approach to disease management that has attracted interest as a potential for ecologically safe disease control and sustainable agriculture.

Numerous scientists have hypothesized that plant pathogens lead to an increase in phenolic chemicals and related defense enzymes in plants (Bhattacharya *et al*. 2010; Baka *et al*. 2013; Mianabadi *et al*. 2015; Mostafa *et al*. 2021a, b 2022). According to some researchers, spraying crops with a lot of plant extracts increased agricultural production (Mvumi *et al*. 2013; Elzaawely *et al*. 2017; Bello *et al*. 2022).

This study attempted to determine how *S. fuliginea*, the cause of cucumber powdery mildew disease, could be controlled using aqueous extracts from five traditional Egyptian medicinal plants. It was also taken into account how these extracts would affect the prevalence and severity of disease both in the lab and in the greenhouse. Additionally, it was confirmed how these extracts affected the phenolic chemicals and associated defense enzymes in cucumber plants.

Materials and Methods

Plant materials and extract preparation

Five traditional Egyptian medicinal plants were purchased from several herb marketplaces (Table 1). For extraction, 1,000 ml of distilled water (DW) (1:10 w/v) was combined with 100 g of each air-dried plant material. Following that, extraction was performed for 24 h in a cool environment. Two sheets of cheesecloth were used to filter plant extracts. The initial concentration for the antifungal activity studies was a 10% aqueous extract concentration.

Effect of aqueous plant extracts (APE) on conidia germination of *Sphaerotheca fuliginea*

Young cucumber leaves with *S. fuliginea* infection were used to harvest the conidia. The conidia were dispersed on dry spotless glass slides containing 1 ml of each APE at various concentrations (2.5, 5, 10, and 20%). As a control, 1 ml of sterile DW was placed on glass slides. The slides were then placed on Petri dishes with sterile DW and lined with wet blotting filter paper. The Petri dishes were then incubated at 25°C for 24 h. Each treatment included five replications. Following incubation, the percentage of conidia germination was assessed for each replicate in ten microscopic fields. However, the percentage of conidia that germinated was calculated using the following formula: percentage of conidia germination = No. of germinated conidia/Total no. of conidia × 100 (Nair and Ellingboe 1962).

Table 1. Traditional medicinal plants used in the present study and parts used

Scientific name	Common name	Family	Traditional medicinal uses	Part used	Author
Citrullus colocynthis (L.) Schrad	Bitter apple	Cucurbitaceae	soothes rheumatism, reduces swelling.	fruit	Aboelsoud (2010)
Curcuma longa L.	Tumeric	Zingiberaceae	closes open wounds (also used to dye skin cloth)	rhizome	Aboelsoud (2010)
Glycyrrhiza glabra L.	Licorice	Fabaceae	mild laxative expels a mild soothes the liver, pancreas, and chest	root	Aboelsoud (2010)
Lawsonia inermis L.	Henna (Egyptian privet)	Lythraceae	astringent stops diarrhea, close open wounds (and used as a dye)	leaves	Aboelsoud (2010)
Tamarindus indica L.	Tamarind	Fabaceae	laxative	fruit	Aboelsoud (2010)

Effect of aqueous plant extracts (APE) on the disease severity of powdery mildew by using the detached leaf technique

A cotton layer wrapped with filter paper and moistened with sterile DW was placed on top of discs (1.5 cm in diameter) of healthy cucumber detached leaves in Petri plates. According to Townsend and Heuberger (1943), *S. fuliginea* conidia suspension on leaf discs was used for inoculation. Ten cucumber leaf discs in Petri plates, measuring 9 cm in diameter, were employed. For each concentration, three Petri dishes containing 30 leaf discs were utilized as replicas. Each treatment was individually sprayed with a different water extract concentration before being infected with the pathogen. Following a 7-day inoculation period, the disease incidence was assessed. In the control treatment, leaf discs were sprayed with DW only. The reduction (%) of disease incidence was calculated in the control experiments.

Greenhouse experiment

Seeds of the pathogen-free local variety *Cucumis sativus* (cv. *Tamra* 761) were obtained from the Department of Vegetable Crop Research, Agricultural Research Centre, Giza, Egypt, and used in this experiment. Seeds were grown in plastic pots with a 15 cm diameter containing 1.5 kg of non-sterile soil (1:1:1 w/w sand, clay, and compost), and 50 ml of 25% Hoagland's solution was supplied 5 days after sowing, and the second application was given 3 days after replanting in pots. All pot experiments were conducted in the greenhouse at the Department of Botany and Microbiology, University of Damietta, Egypt. Plants were watered daily just to satisfy the field capacity but not in excess to drain off freely.

Effect of aqueous plant extracts (APE) on disease severity and incidence

Plants that were 20 days old were treated with water and then given a dusting of *S. fuliginea* conidia by shaking mildewed leaves over the cucumber leaves. One week following the inoculation, plants were sprayed with each of the extracts that had been examined. Only tap water and the fungicide Afugan (also known as triazophos), produced by Bayer Crop Science in Germany were used as controls. After inoculation, plant extracts were sprayed four times at intervals of 7 days. Each treatment and control had four replicates (pots). Inoculated pot plants were kept on wet benches until the disease became apparent. Seven days after spraying, percentages of disease occurrence and severity (disease parameters) were calculated. Disease incidence was calculated as a proportion of infected leaves as a percentage of all leaves on the plant, and disease severity was calculated using the Bock *et al.* (2022) scale. Plants for each particular treatment were classified as follows: completely healthy 1 – 1–2 spots per leaf; 2 – 3–5 spots per leaf; 3 – 6–10 spots per leaf; 4 – up to 25% of leaf area affected; 5 – up to 50% of leaf area affected; 6 – up to 75% of leaf area affected; 7 – greater than 75% of leaf area affected. The percentage of disease severity (DS) for each particular treatment was calculated using the following formula:

$$DS = \text{Sum of } (n \times v)/\text{Total number of observed leaves in sample} \times \text{maximum grading } (7) \times 100,$$

where: n – the number of infected leaves in each category; v – the numerical value of each category. The sum of numerical values was obtained by multiplying the number of leaves (observed in a particular grade) with their respective grades. The percentage of infection was determined on both upper and lower leaf surfaces and averaged.

Determination of total phenol content

According to Waterhouse's approach (2002), total phenol estimation was performed. One g of the leaf material was crushed in 80% ethanol. For 20 minutes, the homogenate was centrifuged at 10,000 rpm. Dryness resulted from supernatant evaporation. The residue was dissolved in 5 ml of DW, and 0.2 ml was placed in a clean test tube. The volume was completed up to 3 ml with DW. Next, 0.5 ml of folin reagent was added. After 3 min, 2 ml of 20% Na_2CO_3 solution was added to every tube. The contents were mixed thoroughly and the tubes were immediately placed in boiling water for just 1 minute and cooled. The absorbance was measured at 650 nm versus the blank. The results were conveyed as gallic acid equivalents (mg of gallic acid/mg of dry weight extract).

Determination of phenolic compounds in *Curcuma longa* rhizome extract by high-performance liquid chromatography (HPLC)

The dried ethanol extract of *C. longa* rhizome was dissolved in ethanol (1 mg · ml^{-1}), filtered, and exposed to an analysis by the Shimadzu HPLC system as described by Zeb (2015). The greatest separation was accomplished in 40 min using a gradient elution of methanol, deionized water, and acetic acid on a Zorbax plus C18 column (4.6 100 mm, 3.5 m) at 25°C. The chromatography peaks were established by comparing their retention times with those of reference standards and by photodiode-array detector (DAD) spectra (200–500 nm).

Estimation of proline

To calculate proline, the method of Senthil-Kumar *et al.* (2021) was employed. As a reagent, 4 ml of syrupy phosphoric acid at a 1:1 dilution and 6 ml of glacial acetic acid were combined with 0.25 g of fully solubilized ninhydrin that was heated to 70°C. Pipetting 1 ml of concentrated water extract into a quick-fit tube, followed by 1.0 ml of glacial acetic acid and 1.0 ml of the reagent at the same time. A reagent blank was also prepared. The samples and blanks were heated at 100°C for 60 min. The tubes were then filled with 1.0 ml of glacial acetic acid and permitted to cool to room temperature. With glacial acetic acid, the volume in every tube was corrected to 5 ml. The optical density of the generated color was determined by spectrophotometric color at 1 h at 515 nm.

Estimation of peroxidase (POD) activity

A mortar and pestle were used to thoroughly mix 40 ml of 0.02 M phosphate buffer (pH 7) with 0.5 g of leaf material. The mixture was then filtered through cheesecloth and centrifuged at 2,000 rpm for 10 min, which was then diluted to 100 ml. All operations were completed at 4°C. Purpurogallin's production caused an increase in absorbance at 420 nm, which was used to measure peroxidase activity. The reaction mixture contained 0.5 ml of 1% H_2O_2, 0.1 ml of enzyme extract, and 3 ml of pyrogallol phosphate buffer (0.05 M pyrogallol in 0.1 M phosphate buffer, pH 6). A unit of an enzyme is defined as 1 g of fresh material · min^{-1}.

Estimation of polyphenol oxidase (PPO) activity

The extract was prepared using the method recommended for peroxidase activity and assayed as the increase in absorbance at 420 nm because of the formation of purpurogallin. The reaction mixture was comprised of 2 ml of 0.02 M phosphate buffer (pH 7), 1 ml of 0.1 M pyrogallol, and 1 ml of enzyme extract. The reaction mixture was incubated for 1 min at 25°C and the reaction was blocked by adding 1 ml of 2.5 N H_2SO_4. One enzyme unit is defined as a unit per g fresh mass per min.

Sample preparation for scanning electron microscopy (SEM)

Sharp razor blades were used to cut away small sections of cucumber leaves that were infected with *S. fuliginea* and then fixed for 24 h at 4°C in 2.5% glutaraldehyde in a buffer solution containing 0.1 M sodium cacodylate and pH 7.2. These tiny particles were then dehydrated through a graduated series to 100% ethanol after being postfixed in 1% OsO_4 in the same buffer and at the same pH. The tissues were then taken out of the ethanol and sliced into several smaller pieces. The fragments were immediately placed back into ethanol, critical point dried with liquid CO_2, mounted on aluminum stubs with silver paint, coated with gold-palladium, and studied under an SEM (JEOL JSM-6400, Japan) at the Electron Microscope Unit, Mansoura University, Egypt.

Determination of fruit yield and its components

Two times every week, cucumber plants in the flowering and fruiting stages were collected. At harvest, each treatment's yield (kg · $plant^{-1}$), number of flowers per plant, number of fruits per plant, average fruit weight, fruit length, fruit diameter, and yield (kg · $plant^{-1}$) were recorded.

Statistical analysis

The results were analyzed using analysis of variance, and the significance was assessed using the least significant difference (LSD) levels of 1 and 5% for each measurement. All measures are the averages of five replicates.

Results

Effect of aqueous plant extracts (APE) on conidia germination of *Sphaerotheca fuliginea*

According to Table 2, all treatments considerably reduced *S. fuliginea*'s conidia germination (%) when compared to the control. By increasing the extract concentrations from 2.5 to 20.0%, the values of conidia germination were dramatically reduced. The lowest *S. fuliginea* conidia germination rate (%) was seen in the extracts with the highest plant concentration (20%). With percentages of 22.05, 25.80, 27.54, 31.46, and 35.32%, respectively, the extracts of *C. longa,* were followed by *Tamarindus indica, Citrullus colocynthis, Glycyrrhiza glabra*, and *Lawsonia inermis* in order of lowest conidia germination.

Effect of aqueous plant extracts (APE) on disease severity and disease incidence of powdery mildew by using detached leaf technique

After being sprayed with water extracts of the tested plants, detached cucumber plant leaves were then cultured for 24 hours with a suspension of *S. fuliginea*

Table 2. Effect of aqueous plant extracts (APE) on conidia germination, 24 h after incubation at 25°C and 100% relative humidity

	Germination % of conidia			
Plant extract conc.	2.5%	5.0%	10.0%	20.0%
Curcuma longa	58.41 ± 0.21	32.70 ± 0.26	28.47 ± 0.43	22.05 ± 0.50
Citrullus colocynthis	65.82 ± 0.23	40.23 ± 0.92	36.42 ± 0.16	27.54 ± 0.43
Lawsonia inermis	76.91 ± 0.34	58.54 ± 0.67	43.41 ± 0.32	35.32 ± 0.21
Tamarindus indica	63.32 ± 0.80	38.45 ± 0.62	30.21 ± 0.74	25.80 ± 0.42
Glycyrrhiza glabra	72.42 ± 0.61	47.42 ± 0.45	40.12 ± 0.23	31.46 ± 0.36
The control	88.45			

± Standard error of the mean

Table 3. Effect of spraying of aqueous plant extracts (APE) on powdery mildew disease incidence on detached leaves, artificially inoculated with *Sphaerotheca fuliginea* conidia

Plant extract conc.	Disease incidence and Disease reduction [%]								Mean of Inc.
	2.5%		5.0%		10.0%		20.0%		
	Inc.	Red.	Inc.	Red.	Inc.	Red.	Inc.	Red.	
Curcuma longa	26.5	67.2	16.6	80.5	13.2	84.1	9.6	90.7	16.5
Citrullus colocynthis	33.1	59.4	26.6	65.2	23.2	70.7	18.5	85.5	25.4
Lawsonia inermis	43.3	48.8	36.7	56.8	23.3	72.4	18.5	70.8	30.5
Tamarindus indica	36.8	56.4	32.6	60.5	24.8	65.3	20.9	75.5	28.8
Glycyrrhiza glabra	53.3	36.9	36.7	56.3	33.2	68.2	30.7	55.6	38.5
The control	82.2								

Inc. – disease incidence; Red. – Disease reduction; reduction relative to the control treatment; LSD – 0.05

conidia. Data in Table 3 demonstrated that the extracts reduced infection rates. By increasing the concentration of extracts from 2.5 to 20.0%, the proportion of detached cucumber leaves that were pathogen-positive was reduced. With water extracts of *C. longa* rhizome, *S. fuliginea* caused disease incidence values of 16.5%, followed by *C. colocynthis, L. inermis, T. indica*, and *G. glabra* as 25.4, 30.5, 28.8, and 38.5%, respectively. The disease reduction was, respectively: 67–90, 59–85, 56–75, 48–70, and 36–55%. It is obvious that *C. longa* rhizome extract significantly reduced the pathogen's ability to cause disease.

Effect of aqueous plant extracts (APE) on disease severity and incidence under greenhouse conditions

Table 4 displays the disease severity – DS (%) of the powdery mildew disease of cucumber plants in the greenhouse trial. Data showed that, compared to the control treatment, all treatments considerably lessened the severity of the disease. According to the results, the severity of the disease varied between 13.1 and 25.07% compared to 53.1% in the control treatment. The water extract of *C. longa* rhizome was the most effective on the disease, followed by *T. indica, C. colocynthis, G. glabra*, and *L. inermis*. They reduced the disease severity by 75.1, 72.2, 65.3, 62.6, and 57.5%, respectively. Generally, the fungicide was more efficient on disease severity than plant extracts, with a reduced value of 87.9%.

Effect of aqueous plant extracts (APE) on total phenol in cucumber leaves infected by *Sphaerotheca fuliginea*

Data in Table 5 showed that, when compared to control healthy plants (12.87 μg · g^{-1} fresh weight) and control diseased plants (13.91 μg · g^{-1} fresh weight), all plant extracts increased total phenols in cucumber leaves. By increasing the concentrations of extracts from 2.5 to 20.0%, the total phenol was considerably ($p \leq 0.05$) increased. The extract of *C. longa* rhizome at a concentration of 20% produced the highest amount of total phenol (25.56 μg · g^{-1} fresh weight), which also represented the lowest proportion of infection and severity. The extract of *L. inermis* at the same concentration yielded the lowest total phenol (16.84 μg · g^{-1} fresh weight), which coincided with the maximum infection and severity.

Table 4. Powdery mildew severity (%) in cucumber treated with aqueous plant extracts in greenhouse experiments

Plant extracts	DS [%]	Red. [%]
Curcuma longa	13.1	75.1
Citrullus colocynthis	21.5	65.3
Lawsonia inermis	25.0	57.5
Tamarindus indica	15.6	72.2
Glycyrrhiza glabra	23.8	62.6
Fungicide	6.4	87.9
The control	53.1	

DS – disease severity; Red. – Disease reduction; Reduction relative to the control treatment; LSD – 0.05

Table 5. Effect of spraying of plant extracts on total phenols in cucumber leaves infected by *Sphaerotheca fuliginea*

Total phenol [µg · g^{-1} fresh weight]				
Plant extract conc.	2.5%	5.0%	10.0%	20.0%
Control (healthy)		12.87		
Control (infected)		13.91		
Curcuma longa	18.45	19.31	22.24	25.56
Citrullus colocynthis	16.31	16.82	17.45	18.47
Lawsonia inermis	14.32	15.10	15.98	16.84
Tamarindus indica	17.24	18.21	20.15	22.84
Glycyrrhiza glabra	15.25	15.98	16.76	17.82

Table 6. Effect of spraying of plant extracts on proline content in cucumber leaves infected by *Sphaerotheca fuliginea*

Proline content [µg · g^{-1} fresh weight]				
Plant extract conc.	2.5%	5.0%	10.0%	20.0%
Control (healthy)		13.5		
Control (infected)		14.9		
Curcuma longa	18.45	19.31	22.24	25.56
Citrullus colocynthis	16.31	16.82	17.45	18.47
Lawsonia inermis	14.32	15.10	15.98	16.84
Tamarindus indica	17.24	18.21	20.15	22.84
Glycyrrhiza glabra	15.25	15.98	16.76	17.82

Table 7. Effect of spraying of plant extracts on peroxidase (POD) activity in cucumber leaves infected by *Sphaerotheca fuliginea*

Peroxidase activity [Umin · g^{-1} fresh weight]				
Plant extract conc.	2.5%	5.0%	10.0%	20.0%
Control (healthy)		12.5		
Control (infected)		13.9		
Curcuma longa	18.4	19.8	22.7	28.9
Citrullus colocynthis	16.8	16.9	18.2	20.5
Lawsonia inermis	14.1	14.8	15.6	18.9
Tamarindus indica	17.3	17.9	19.5	22.8
Glycyrrhiza glabra	15.7	16.8	17.5	19.8

Effect of aqueous plant extracts (APE) on proline content in infected cucumber leaves

Data presented in Table 6 revealed that the infection of cucumber plants by *S. fuliginea* resulted in a significant increase in proline content (14.9 µg · g^{-1} fresh weight) in comparison to the healthy control plants (13.5 µg · g^{-1} fresh weight). Treatment with the extracts at different concentrations from tested plants caused an increase in proline content. The extract of *C. longa* rhizome at a concentration of 20% displayed the highest proline content (25.56 µg · g^{-1} fresh weight).

Effect of aqueous plant extracts (APE) on peroxidase (POD) content in infected cucumber leaves

Data presented in Table 7 revealed that the activity of POD was significantly increased ($p \leq 0.05$) by increasing the concentrations of extracts from 2.5 to 20.0% when compared with the healthy (12.5 Umin · g^{-1} fresh weight) and infected (13.9 Umin · g^{-1} fresh weight) controls. The highest level of POD (28.9 Umin · g^{-1} fresh weight) was obtained with the aqueous extract of *C. longa* rhizome at a concentration of 20%, which also reflected the lowest percentage of infection and severity. The lowest level of POD (18.9, 3.0 Umin · g^{-1} fresh weight) was obtained with the extract of *L. inermis* at the same concentration and was accompanied by the highest infection and severity.

Effect of aqueous plant extracts (APE) on polyphenol oxidase (PPO) content in infected cucumber leaves

Data presented in Table 8 showed that the activity of PPO was significantly increased ($p \leq 0.05$) by raising the concentrations of extracts from 2.5 to 20.0% when compared to the healthy (1.12 Umin · g^{-1} fresh weight) and infected (1.20 Umin · g^{-1} fresh weight) controls. The uppermost level of PPO (6.86 Umin · g^{-1} fresh weight) was discovered with the aqueous extract of *C. longa* at a concentration of 20%, which also reflected the lowest percentage of infection and severity. The lowest level of PPO (3.00 Umin · g^{-1} fresh weight) was gained with the extract of *L. inermis* at the same concentration and conveyed the highest infection and severity.

Table 8. Effect of spraying of plant extracts on polyphenol oxidase (PPO) activity in cucumber leaves infected by *Sphaerotheca fuliginea*

Polyphenol oxidase activity [Umin · g^{-1} fresh weight]				
Plant extract conc.	2.5%	5.0%	10.0%	20.0%
Control (healthy)		1.12		
Control (infected)		1.20		
Curcuma longa	2.30	2.81	4.52	6.86
Citrullus colocynthis	1.98	2.10	2.90	3.65
Lawsonia inermis	1.23	1.62	2.31	3.00
Tamarindus indica	2.10	2.45	3.24	5.42
Glycyrrhiza glabra	1.30	1.80	2.53	3.20

High-performance liquid chromatography (HPLC) analysis of phenolic compounds of *Curcuma longa* rhizome extract

The peak of caffeic acid hexoside appeared in the HPLC chromatogram of the *C. longa* rhizome extract at a retention time of 1 min (peak 1), followed by those of curdione at 8.5 min (peak 2), diallyl-hexoside at 10.7 min (peak 3), sinapic acid at 13.5 min (peak 4), and demethoxycurcumin at 14.8 min (peak 5). In addition, valoneic acid bilactone at 16.9 min (peak 6), coumaric acid at 22.6 min (peak 7), curcumin at 25.8 min (peak 8), and quercetin-3-D-galactoside at 27.1 min (peak 9) were also recorded. Furthermore, bisdemethoxycurcumin at 28.7 min (peak 10), caffeic acid at 29.3 min (peak 11), curcuminol at 30.6 min (peak 12), and curcumin-O-glucuronide were estimated (Table 9). The concentration of pure curcumin was 3220.8 μg · g^{-1}, followed by demethoxycurcumin (2312.8 μg · g^{-1}), and curcumin-o-glucuronide (2150 μg · g^{-1}), which had the next-highest amount at Rt of 25.8 min. Demethoxycurcumin was second with a concentration of 2312.8 μg · g^{-1}, followed by curcumin-O-glucuronide with a concentration of 2150 μg · g^{-1}.

Effect of aqueous plant extracts (APE) on cucumber yield and its components

The impact of various *C. longa* rhizome extract concentrations on the yield and its constituent parts (number of flowers/plant, number of fruits/plant, mean weight of fruit, fruit length, fruit diameter, and yield/plant) is displayed in Table 10. All concentrations caused all metrics to increase in comparison to the control. The highest extract concentration boosted the cucumber plants' output. In comparison to the control and low concentrations (2.5–10%), the yield (at 20%) was 2.01 kg · plant^{-1}.

Scanning electron microscopic studies

Untreated infected cucumber leaves were found to have extensive surface mycelia, a profusion of conidiophores, and chains of growing conidia. The conidia had a regular form and smooth surfaces (Fig. 1 A), and each conidium had one germ tube growing out of it

Table 9. Identification and quantification of phenolic compounds in *Curcuma longa* rhizome extract

Peak	Rt	Compound	Absorption spectra	Concentration [μg · g^{-1}]
1	1	caffeic acid hexoside	287, 231	156.4 ± 3.9
2	8.5	curdione	302, 280	210.2 ± 2.8
3	10.7	digalloyl-hexoside	365, 225	389.2 ± 4.9
4	13.5	sinapic acid	325, 287	12.9 ± 2.0
5	14.8	demethoxycurcumin	419, 278	2.312.8 ± 11.4
6	16.9	valoneic acid bilactone	376, 268	1.092.8 ± 8.9
7	22.6	coumaric acid	308, 280	125.5 ± 7.8
8	25.8	curcumin	432, 256	3.220.8 ± 21.4
9	27.1	quercetin-3-D-galactoside	355, 251	9.7.6 ± 1.7
10	28.7	bisdemethoxycurcumin	417, 255	248.1 ± 6.9
11	29.3	caffeic acid	322, 287	65.1 ± 3.8
12	30.6	curcuminol	425, 281	176.5 ± 2.5
13	31.2	curcumin-O-glucuronide	425, 267	2.150.4 ± 11.2
14	35.3	isorhamnetin	375, 253	1.658.7 ± 13.8
15	37.1	casuarinin	357, 263	584.8 ± 6.4
16	37.9	ferulic acid	365. 240	20.6 ± 2.1
17	38.4	vanillic acid	318, 210	8.9.5 ± 1.8

– Standard error of mean±

Table 10. Effect of aqueous extract of *Curcuma longa* rhizome on cucumber yield and its components

Extract conc.	Yield and its components					
	no. of flowers/plant	no. of fruits/plant	mean weight of fruit [gm]	fruit length [cm]	fruit diameter [cm]	yield [kg · plant^{-1}]
Control	38.34 ± 3.1	12.90 ± 0.9	75.34 ± 9.7	10.12 ± 0.7	2.71 ± 0.5	0.90 ± 0.2
2.5%	20.27 ± 2.2	9.56 ± 0.8	81.31 ± 8.4	12.43 ± 0.8	2.88 ± 0.6	1.12 ± 0.4
5.0%	30.31 ± 2.5	10.32 ± 1.7	90.64 ± 11.4	13.20 ± 1.1	3.51 ± 0.1	1.66 ± 0.5
10.0%	38.62 ± 3.4	12.87 ± 0.9	92.21 ± 11.5	14.86 ± 2.3	3.98 ± 0.7	1.63 ± 0.6
20.0%	46.21 ± 3.5	15.82 ± 1.0	99.72 ± 13.6	18.80 ± 2.1	5.21 ± 0.9	2.01 ± 0.8

± – Standard error of mean

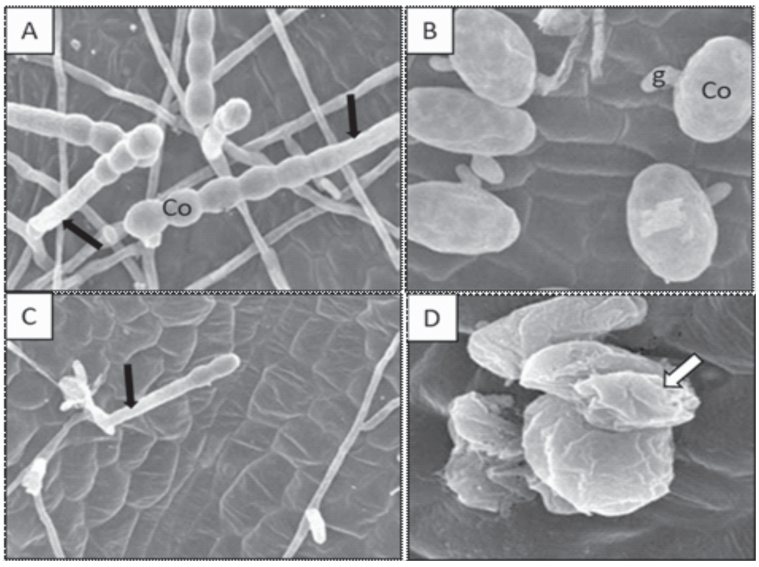

Fig. 1. A and B – untreated infected cucumber leaves. A – dense surface mycelia and abundant conidiophores (black arrows) with developing conidia in chains (Co). Scale Bar = 20 µm; B – normal conidia (Co) with a smooth surface and one germ tube (g) emerging from one conidium. Scale Bar = 10 µm; C and D – treated infected cucumber leaves with 20% of *Curcuma longa* rhizome extract. C – small colonies with fewer mycelia and immature conidiophores (black arrow) carrying immature conidia. Scale Bar = 50 µm; D – swollen and ruptured conidia (white arrow) with wrinkled surfaces. Scale Bar = 10 µm

(Fig. 1B). In contrast, infected cucumber leaves when treated with the 20% *C. longa* rhizome extract revealed tiny colonies with fewer mycelia and immature conidia (Fig. 1 C). The conidia had wrinkled and torn surfaces and seemed bloated (Fig. 1 D).

Discussion

The goal of the current research was to lessen the usage of chemicals in agricultural processes and identify the best non-chemical strategy for shielding cucumber plants from the powdery mildew disease brought on by *S. fuliginea*. The use of chemical pest management is troublesome due to the emergence of disease resistance and issues with environmental pollution. Pesticide residues must be avoided or kept to a minimum in cucumber products that are intended for sale. Therefore, the development of alternative control methods based on plant extracts, especially medicinal plants, which are more widely available, and less expensive is crucial (Baka 2010). According to Al Surhanee (2013), medicinal plant extracts are effective at preventing powdery mildew in greenhouses and testing facilities.

The current findings demonstrated that *S. fuliginea*'s conidia germination was decreased in laboratory testing by the water extracts of the medicinal plants studied. The natural bioactive chemicals found in the studied extracts may be the cause of their inhibitory impact (Baka 2014a, b). The pathogen's conidia germination was most effectively inhibited by an extract from the rhizomes of *C. longa*. Due to its therapeutic uses, the genus *Curcuma* has been in use for a long time.

The detached leaf method demonstrated that the aqueous plant extracts of all examined plants reduced *S. fuliginea* disease. Reports on the effectiveness of

plant extracts in preventing powdery mildew disease exist. The results of the current studies demonstrated that the powdery mildew disease severity was greatly decreased by the water extracts of the investigated medicinal plants. The use of *C. longa* rhizome extract in greenhouse applications produced the best reductions in the severity of the powdery mildew disease. This might be a result of the extract's high curcumin level. These results are revealed in an ambitious study on the use of medicinal plant extracts in the prevention of fungal plant diseases, which successfully controlled powdery mildew in tests conducted in greenhouses and laboratories (Al Surhanee 2013).

Plants with a high content of polyphenols are resistant to several plant diseases. Phenols have been shown to provide resistance to diseases and pests in plants. Numerous studies have found that plant resistance to various fungal infections was positively correlated with the amount of phenolic content, which was found to be important (Abo-Elyousr *et al*. 2009).

Using HPLC, the primary phenolic components that might be crucial in the inhibition of the tested pathogen were identified in the crude extract of *C. longa* rhizome. The results that were given validated the existence of several efficient composites, for example, curcumin, which was present in the highest concentration in the *C. longa* rhizome extract (3220.8 $\mu g \cdot g^{-1}$). Plants produce phenolic chemicals, which are aromatic benzene rings containing one or more hydroxyl groups, mostly as a kind of stress relief (Bhattacharya *et al.* 2010). Additionally, phenolics, which are widely distributed in most plants and have a vital role in plant resistance to many diseases, are the primary unit of secondary metabolites (Farkas and Kiraaly 2008).

Furthermore, they serve as insecticides and defense mechanisms against fungi (Lattanzio *et al.* 2006). Plants accumulate a large amount of simple and complex phenolics, which function as phytoalexins and phytoanticipins against plant diseases (Kumar *et al.* 2020). Therefore, it has been proposed that phenolic compounds could serve as viable alternatives for the chemical control of plant diseases in agriculture (Mostafa *et al*. 2021b). Plants collect phytoalexins such as hydroxycoumarins and hydroxycinnamate conjugates in response to pathogen attacks (Iravani 2011).

It was demonstrated that curcumin enhanced the effectiveness of popular azole and polyene antifungal (Sharma *et al.* 2010). This study is the first to examine how curcumin significantly reduced the growth of *S. fulginea*, though Fu *et al*. (2018) discovered that (+)-(S)-ar-turmerone, another bioactive component of *C. longa*, was also effective against *S. fuliginea*. According to Fuloria *et al*. (2022), *Aspergillus flavus*, *A. parasiticus*, *Fusarium moniliforme*, and *Penicillium digitatum* are all susceptible to the antifungal effects of *C. longa* oil extracted by ether, and chloroform. Changes in ergosterol synthesis, proteinase secretion, or membrane-associated ATPase activity may be responsible (Martins *et al*. 2009). When compared to the untreated control in the current investigation, the application of plant extracts to infected cucumber leaves caused an excessive buildup of proline.

Proline is a multi-functional amino acid that gives tolerance to plants against abiotic and biotic stressors and has been linked to plant defense against infections (Szabados and Savoure 2010; Senthil-Kumar and Mysore 2012). In response to a variety of biotic and abiotic stressors, many plants frequently accumulate proline in their tissues. Depending on the species, proline accumulation levels in plants can be up to 100 times higher than they would be in a controlled environment (Verbruggen and Hermans 2008). The intermediate byproduct of both proline production and catabolism is pyrroline-5-carboxylate (P5C). According to Qamar *et al*. (2015), proline-P5C metabolism is closely controlled in plants, especially when they are under biotic and abiotic stress.

The current results also showed that cucumber plants' total phenol levels and related defense enzymes like PDP and PPO were increased against powdery mildew disease in response to the tested medicinal plant extracts. These findings concur with those made public by Baka *et al.* (2013). This rise in total phenol may have been caused by an improvement in plant defenses against infectious diseases and growth. Plants include a variety of phenolic chemicals that prevent lipid peroxidation and stabilize free radicals (Newairy and Abdou 2009). According to Zhang *et al*. (2022), the buildup of phenolic compounds in infected plant tissues may improve host resistance by promoting host defense mechanisms and limiting the spread of fungal growth in plant tissues (Were *et al*. 2022).

By using oxygen and creating fungitoxic quinones, the oxidative processes catalyzed by the PPO and POD and made possible by the increased phenolic content render the environment unfavorable for the continued growth of pathogens. The necrotic process, such as the oxidative polymerization involving phenolic chemicals, amino acids, and proteins that produce brown melanin, may also be connected to the antifungal activity of oxidized phenolics. The outcome of this reaction is the establishment of an impermeable barrier to plant parasite pathogenesis and a reduction in nutrients necessary for fungal development (Beckman *et al.* 1974). It is generally known that plants produce phenols early in response to pathogen infection attempts in the form of antifungal compounds, signal molecules, and components that strengthen cell walls (Kruger *et al*. 2002).

In comparison to the control, the foliar application of *C. longa* rhizome extract considerably increased the

cucumber fruit yield and its constituent parts. It was shown that the highest increase in cucumber yield and its constituents resulted from the high concentration of *C. longa* rhizome extract (20%). Many researchers have found that spraying crops with an effective dosage of plant extracts enhanced the production of many crop plants (Mvumi *et al*. 2013; Elzaawely *et al*. 2017; Bello *et al*. 2022). This is the first time, to my knowledge, that *C. longa* rhizome extract has been utilized to improve cucumber plants' production and its constituent parts.

Additionally, 20% *C. longa* rhizome extract treatment of infected cucumber leaves revealed abnormalities in the pathogen's growth, notably the conidia as observed by SEM. This might be the result of the cytoplasm retracting in the hyphae and conidia, which finally results in the mycelium dying (Sharma and Tripathi 2008). These alterations are brought about by plant extract components interfering with the enzymatic processes of wall formation, which affects fungal morphogenesis and growth (Alotibi *et al*. 2020).

Conclusions

The current study demonstrated and supported the hypothesis that *C. longa* rhizome extract could be used as an efficient, safe, and natural bio-stimulant to decrease the use of synthetic agrochemicals in organic agricultural production and, as a result, replace risky chemicals with safe ones in the production of high-quality vegetables. The use of plant extracts has a bright future, especially extracts from the curcumin-rich *C. longa* species. These plant extracts can be used to control fungus-based plant diseases for less money and with less environmental risk. The usage of plant extracts that are efficient against fungal plant pathogens could be used to create novel, inexpensive antifungal drugs due to the scarcity and high cost of natural antifungal agents. Although ethanolic extracts were the most successful in the literature, aqueous extracts are advised.

Acknowledgments

The author would like to thank the University of Damietta, Egypt for financial support to complete this work.

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