

Observation of Powdery Mildew on Safflower in Egypt, and its Control Using Propolis, Diluted Honey and Clove Essential Oil

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ABSTRACT

Powdery mildew has recently become a serious problem threatening safflower in its growing areas in Egypt. The disease mainly attacks leaves and stems, and infection may extent to inflorescences, causing heavy losses. The disease was widespread in the areas surveyed. However, its severity varied between these regions, as it was severe in Qena, followed by Beni-Suef and less severe in Fayoum. Based on the morphological characteristics of the anamorphic stage, the causal agent of safflower powdery mildew (SPM) was identified as *Golovinomyces cichoracearum* (DC.) V.P. Heluta. In this study, we tested the activity of ethanolic propolis extract (EPE), diluted honey solution (DHS), and clove essential oil (CEO), individually or in mixtures against SPM in *in vitro* and *in vivo*. All treatments significantly reduced conidial germination of *G. cichoracearum in vitro*. Maximum reduction was achieved by EPE (50%) + CEO (3 mL/L), followed by EPE (50%) + DHS (50 g/L) and DHS (50 g/L) + CEO (3 mL/L) as follows: 90, 87.3, and 80.1%, respectively. While the lowest reduction was recorded by DHS (50 g/L). In the greenhouse, all treatments significantly reduced disease severity and area under the disease progress curve (AUDPC). The mixture treatments were more efficient than the individual treatments. Similar results were obtained in the field during two successive seasons. The reduction in disease severity was expressed by improved growth, yield, photosynthetic pigments, and anatomical characteristics of the plant. In general, EPE, DHS, and CEO can be successfully used to control powdery mildew and improve growth and productivity in safflower.

KEYWORDS: *Golovinomyces cichoracearum*, safflower, propolis, diluted honey, clove essential oil, anatomical structures

1. INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is an annual or biennial herbaceous plant belonging to the Asteraceae family. It is mainly cultivated to obtain seeds to extract oil rich in polyunsaturated fatty acids, as well as flowers rich in the orange-red pigment (carthamin) (Zhang et al., 2016). Safflower is widespread in India, China, Egypt, and Iran (Jinous and Nastaran, 2013). In Egypt, its cultivation is mainly distributed in the governorates of Assiut, Qena and Aswan (Ahmed et al., 2016). Safflower is affected by many fungal diseases, causing reduced productivity and increased production costs (Mahadik and Mali, 2018). Among these diseases is powdery mildew, which attacks stems, leaves and even inflorescences, causing a sharp decline in yield (Saluja and Bhide, 1963; Siddaramaiah et al., 1981). Powdery mildew is usually controlled by different classes of fungicides, such as propiconazole, azoxystrobin, and tebuconazole. However, their widespread and unregulated use has led to the emergence of fungicide-resistant isolates, as well as contamination of the environment and food sources (Ahmed et al., 2021). For these reasons, more balanced, effective, and eco-friendly approaches for managing such diseases must be implemented. Recently, natural substances derived from medicinal plants such as clove essential oil, or produced by beneficial insects such as propolis and bee honey, have attracted the attention of researchers due to their bioactive compounds known for their strong antifungal, antibacterial, and insecticidal activity (Hannan et al., 2004; El-Saber Batiha et al., 2020).

Propolis (bee glue) is a brownish resinous substance collected by honey bee workers (*Apis mellifera*) from the leaf buds and cracks of many tree species such as birch, pine, poplar, alder, palm, and willow (Park et al., 2004). Propolis is used to cover hive cracks in order to strengthen its walls, regulate temperature and humidity, and protect it from microbial invasions (Anjum et al., 2019). The chemical composition of propolis includes more than 300 bioactive compounds, such as phenolic compounds, alcohols, esters, aldehydes, ketones, steroids, terpenes, coumarins, vitamins, amino acids, fatty acids, inorganic substances and enzymes (Kustiawan et al., 2017). These

components vary according to the geographical region, environmental factors, plant source, season, and harvest period (Anjum et al., 2019). Numerous reports have proved potent antifungal activity of propolis against plant pathogenic fungi under *in vitro* conditions. Among these studies is Er (2021), who found that ethanol and watery extracts of propolis had high inhibitory effects of up to 97% against the mycelial growth of *Alternaria brassicicola*, *Verticillium dahliae*, *Fusarium graminearum*, and *Pythium ultimum*. Similarly, Çakar et al. (2022) tested the inhibitory activity of three types of propolis extracts against mycelial growth of *F. solani*. The results revealed that the pure extract was the most effective, followed by the ethanol extract, while the DMSO (dimethyl sulfoxide) extract was the least effective. In a similar context, several reports have documented the successful use of propolis to control plant diseases *in vivo*. For example, treatment of bean, grapevine, cucumber, and strawberry with propolis extract reduced fungal diseases caused by *Botrytis cinerea*, *Rhizoctonia solani*, *Podosphaera fuliginea*, *Sclerotium rolfsii*, and *Penicillium digitatum* (Abd-El-Kareem et al., 2018). Also, Heck et al. (2015) found that treatment of cucumber with propolis extract (8%) significantly reduced powdery mildew severity and AUDPC. Moreover, a mixture of propolis with gum Arabic at 1.5% or with chitosan at 5% significantly reduced postharvest diseases in papaya fruits and maintained their quality during storage (Barrera-Necha et al., 2015).

Bee honey is a natural product of honey bees that mainly contains monosaccharides, disaccharides, and oligosaccharides (Shin and Ustunol, 2005). It is also a source of important minerals, proteins, lipids, enzymes, organic acids, phenolic compounds, and inorganic acids (Saxena et al., 2010). Exogenous application of diluted honey solution has been found to stimulate some physiological and biochemical changes in the plant, as well as increasing water and nutrient uptake and reducing harmful excess reactive oxygen species (ROS) (Semida et al., 2019). In addition to enhancing resilience against different stresses (Rouphael and Colla, 2018). Many studies have proved the antibacterial activity of diluted honey solution against a number of pathogenic bacteria, including around 60 species of aerobic,

anaerobic, Gram-positive, and Gram-negative bacteria. Among these bacteria are *Bacillus cereus*, *Acinetobacter baumannii*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Micrococcus luteus*, *Shigella flexneri*, *Serratia marcescens*, and *Staphylococcus aureus* (Hannan et al., 2004; Hegazi et al., 2017). Despite the strong antibacterial activity of diluted honey solution, our knowledge of its antifungal activity is still limited.

Essential oils (EOs) are one of the promising alternative strategies not only for plant disease control but also for low toxicity to humans and safe environment (Moumni et al., 2021). EOs are a complex mixture of secondary metabolites that possess several biological properties (Bakkali et al., 2008). Due to their antimicrobial activities, they have been successfully exploited in integrated pest management against fungi, bacteria, viruses and nematodes (Dorman and Deans, 2000). One of these EOs is clove EO, which is extracted from the flower buds and leaves of clove (*Syzygium aromaticum* L.). It contains many phenolic compounds known for their antifungal, antibacterial, and insecticidal activity (El-Saber Batiha et al., 2020). Extensive reports have shown the efficacy of clove EO in controlling plant pathogens. For example, Ahmed et al. (2023a) demonstrated that clove EO at 1 mL/L completely reduced mycelium growth of *S. sclerotiorum* and *F. solani* *in vitro*, it also significantly reduced root rot and wilt of marigold when used as a seed soak (3 mL/L) and foliar spray (1.5 mL/L). Similarly, Sharma et al. (2017) found that clove EO was most efficient in reducing mycelium growth and conidial germination of *F. oxysporum* f. sp. *lycopersici*. Also, Abdel-Kader et al. (2011) found that

coating the seeds of bean with clove EO significantly reduced root rot in the pre and post emergence stages. This study aims to (1) identify the causal organism of SPM; (2) investigate the activities of ethanolic propolis extract, diluted honey solution, and clove EO, individually or in mixtures against the disease *in vitro* and *in vivo*; and (3) measure the effect of treatments on growth, yield, and plant anatomy.

2. MATERIALS AND METHODS

2.1. Plant and Soil Materials, Treatments and Experimental Site

This investigation was conducted in the laboratory, greenhouse, and experimental farm of Sids Agric. Res. Station, ARC, Beni-Suef governorate, during the 2022/2023 and 2023/2024 seasons, to identify safflower powdery mildew (SPM) and evaluate the efficacy of ethanolic propolis extract, diluted honey solution, and clove essential oil, individually or in a mixture to control this disease. The treatments tested are listed in detail in Table 1. Safflower seeds (cv. Giza 1), propolis, clover honey, and clove essential oil were provided by the Horticultural Crop Techno. Lab., Nat. Res. Center, Egypt. The seeds were sown in the greenhouse and field on October 1st. Soil analysis was performed at the Central Fertilizer Analysis Laboratory, Soil, Water and Environ. Res. Inst., ARC, Egypt, by the methods of Özbek et al. (1995). The soil was clay, and the values of clay, silt, and sand were 55.2, 30.7, and 14.1%, respectively, pH = 7.7, EC = 1.5 ds/m, and organic matter (1.25%). The N, P, and K values were 40.6, 7.0, and 133 mg/kg soil, respectively.

Table 1. Details of the treatments used to control safflower powdery mildew (SPM) in the current investigation.

Treatment No.	Description of Treatments	Rate Used
1	Ethanolic propolis extract (EPE)	50%
2	Diluted honey solution (DHS)	50 g/L
3	Clove essential oil (CEO)	3 mL/L
4	EPE + DHS	50% + 50 g/L
5	EPE + CEO	50% + 3 mL/L
6	DHS + CEO	50 g/L + 3 mL/L
7	Micronized sulfur	2.5 g/L
8	Control (water only)	–

2.2. Observing and Surveying Safflower Powdery Mildew (SPM)

In the winter season of 2021/2022, symptoms of SPM appeared for the first time in a private field in Beni-Suef governorate, Egypt. The disease survey was conducted monthly during the period from November to April in three Egyptian governorates: Beni-Suef, Fayoum, and Qena. Plants were randomly examined in each area by measuring disease incidence and severity.

2.3. Collecting Samples, Isolating and Identifying Pathogenic Fungus

Powdery mildew-infected safflower plants were collected from naturally infected fields in Beni-Suef governorate. Microscopic preparations were made by placing epidermal strips taken from affected parts on glass slides, staining them (methylene cotton blue) and examining them by light microscopy (Olympus BX60, Japan) at 10, 20, and 40 \times . The fungus was identified morphologically by examining the shape and size of conidiophores, conidia and foot cells. Identification was performed at the Department of Fungi and Plant Diseases Survey, Plant Pathol. Res. Inst., ARC.

2.4. Pathogenicity, Fungal Inoculum Preparation and Inoculation

Powdery mildew-infected safflower samples were collected as above. Pathogenicity was confirmed by shaking infected parts over five healthy 45-day-old seedlings (cv. Giza 1). Five uninoculated plants were kept as a control. Inoculated and control plants were placed in two separate moist chambers for 24 h (at 25 \pm 2 $^{\circ}$ C and 70% RH) to promote infection. Disease development was evaluated 30 days post inoculation. To prepare the fungal inoculum, conidia were scraped off with a sterile brush and suspended in 100 mL of sterile distilled water, then two drops of Tween-20 were added. The mixture was centrifuged for 5 min at 3000 rpm and then adjusted to 5 \times 10⁵ spores mL⁻¹ (Kitao and Doazan, 1989). Inoculation was performed by spraying the spore suspension onto 45-day-old healthy plants.

2.5. In Vitro Preparations

2.5.1. Ethanolic Propolis Extract (EPE)

Ten g of propolis powder was dissolved in 100 mL of ethanol (70%), then the mixture incubated for 14 days at 37 $^{\circ}$ C. The mixture was filtered by Whatman filter paper (No. 1) and then centrifuged at 150 rpm for 72 h. The resulting filtrate was considered 100%, and through dilution with sterile distilled water, a 50% concentration was prepared. The chemical composition of Egyptian propolis used in the current study is presented in Table 2.

Table 2. Chemical components of Egyptian propolis used in the present investigation.

Component	Value	Component	Value
Moisture (%)	8.11	Fibers (%)	49.08
Carbohydrates (%)	7.10	Volatile substances (%)	4.20
Ash (%)	2.05	Insoluble matter (%)	38.55
Resin (%)	60.32	Total alkaloid (g/100 g FW)	5.31
Proteins (%)	10.42	Total phenolic content (mg/GAE g DW)	264.11
Fats (%)	19.63	Total flavonoids (mg/qu. g DW)	73.90

2.5.2. Diluted Honey Solution (DHS)

About 50 g of clover honey was dissolved in one liter of tap water. The resulting solution was used 20 min after preparation. The honey used in this study was analyzed at the Beekeeping Res. Dept., Plant Protection Res. Inst., ARC, Egypt, for its active ingredients as shown in Table 3. Moisture, proline, and pH were estimated (AOAC, 1995). Mineral

elements were determined according to the methods of Chapman and Pratt (1961). Quantities of sugars (glucose, sucrose, maltose, and fructose) were assessed by HPLC (High Performance Liquid Chromatography) (Bogdanov and Baumann, 1988). Ascorbic acid was determined (Mukherjee and Choudhuri, 1983). Antioxidant activity was estimated using DPPH assay (1,1-diphenyl-2-picrylhydrazyl) according to Lee et al. (2003).

Table 3. Chemical composition of raw clover honey used in the current study (based on fresh weight of sample).

Component	Value	Component	Value
Moisture (%)	16.1	Sugar types (%):	–
pH	3.7	Glucose	24.8
Minerals (mg/kg FW):	–	Sucrose	6.2
Potassium (K)	357.4	Maltose	3.9
Calcium (Ca)	80.3	Fructose	44.7
Zinc (Zn)	5.9	Antioxidant substances:	–
Phosphorus (P)	51.5	Ascorbic acid (mg/kg FW)	15.8
Magnesium (Mg)	73.3	DPPH radical-scavenging activity (%)	79.2
Copper (Cu)	3.5	Osmoprotectant substances:	–
Iron (Fe)	58.9	Proline (mg/kg FW)	51.2
Iodine (I)	82.4	Total soluble sugars (%)	74.3
Manganese (Mn)	9.5		

2.5.3. Clove Essential Oil (CEO)

The CEO was extracted according to the method of Phu et al. (2019). 200 g of dry flower-bud powder of clove was hydro-distilled in 1000 mL of distilled water for 6 hours. The resulting EO was passed over Na₂SO₄ to eliminate water, and then placed in dark vials. A known amount of EO mixed with 2 mL of Tween 20 was added to sterile distilled water, then the volume was supplemented to 100 mL to obtain the required concentrations (Ko et al., 2003).

2.6. Antifungal Effect of EPE, DHS and CEO on Conidia Germination of *G. cichoracearum*

The antifungal effect of EPE, DHS, and CEO, individually or in mixtures, on conidia germination of *G. cichoracearum* was investigated. Conidia were scraped from infected safflower leaves with a sterile brush and placed on clean glass slides (Nair et al., 1962). Two drops of the tested concentration were applied to the spores, and the slides were then placed on glass pieces in sterile petri dishes (9 cm d.) containing moistened sterile paper. Spores treated with sterile distilled water were considered as a control. Plates were incubated for 24 or 48 h, at 25 ± 2 °C. Three replicates were used for each treatment and five dishes for each replicate. Conidial germination (CG) and reduction (R) were calculated as follows:

$$CG \% = (A/B) \times 100$$

$$R \% = (CG \text{ in C} - CG \text{ in T} / CG \text{ in C}) \times 100$$

Where A = no. of germinated conidia, B = total no. of conidia, C = control, and T = treatment.

2.7. In Vivo Experimental Design

2.7.1. Greenhouse Trials

Greenhouse trials were designed to evaluate the efficacy of EPE, DHS, and CEO, individually or in mixtures, to control SPM, using CRBD (a completely randomized block design). Safflower seeds were sown in pots (30 cm diameter), pre-filled with sterilized sandy loam soil (1:3 w/w) and thinned to one plant/pot. After 45 days of sowing, inoculation was performed with spore suspension (5×10^5 spore mL⁻¹). Two days after inoculation, treatments were sprayed three times at an interval of 15 days. The severity of SPM was assessed 7, 14, 21, and 28 days after the last application, and then AUDPC was calculated.

2.7.2. Field Trials

During the 2022/2023 and 2023/2024 seasons, two field trials were carried out to evaluate the effectiveness of EPE, DHS and CEO, individually or in mixtures against SPM. Experiments were arranged in CRBD, with 3 replicates. Experimental plot consisted of 4 rows (4 × 0.7 m). Safflower seeds were sown in hills spaced 30 cm apart. Plants were allowed to become infected with powdery mildew naturally, and as soon as the first symptoms of the disease appeared, treatments were applied and the severity of the disease was then measured.

2.8. Experimental Measurements

2.8.1. Disease Evaluation

The disease severity (DS) of SPM was classified into five grades: 0 (no infection observed), 1 (1-5%), 2 (6-25%), 3 (26-50%), and 4 (more than 50%) of the affected leaf area, according to Morishita et al. (2003) scale. Disease severity was measured by the next equation:

$$DS \% = \sum (n \times v) / 5 N \times 100$$

Where n = no. of diseased leaves in grade, v = grade numerical values, and N = total no. of diseased leaves. The disease incidence of SPM was assessed using the next equation:

$$\text{Disease incidence \%} = (n/N) \times 100$$

Where n = no. of diseased leaves and N = total no. of leaves examined. Disease reduction (R) was calculated using the next equation:

$$R \% = (DS \text{ in C} - DS \text{ in T} / DS \text{ in C}) \times 100$$

Where C = control and T = treatment. The area under the disease progress curve (AUDPC) was assessed using the next equation (Pandey et al., 1989):

$$AUDPC = D [1/2 (Y_1 + Y_k) + Y_2 + Y_3 + \dots + Y_{k-1}]$$

Where D = time intervals between consecutive records, Y₁ = first disease score, Y_k = last disease score, and Y₂, Y₃, Y_{k-1} = intermediate disease scores.

2.8.2. Growth and Yield Characteristics

At the time of safflower harvest, a number of growth factors, including plant height (cm) and number of leaves/plant were assessed along with a number of yield characteristics such as number of heads/plant, number of seeds/plant, and weight of 100 seeds (g).

2.8.3. Photosynthetic Pigments

Chlorophyll a, chlorophyll b, and carotenoids were estimated according to the method of McLeroy-Etheridge and McManus (1999) using spectrophotometer. Pigments were extracted from safflower fresh leaves in aqueous acetone (85%: v/v). Absorbance was read at 452.5, 644, and 663 nm. The pigment content was estimated as follows:

$$\text{Chl. a (mg mL}^{-1}\text{)} = 10.3 \times E_{663} - 0.918 \times E_{644}$$

$$\text{Chl. b (mg mL}^{-1}\text{)} = 19.7 \times E_{644} - 3.87 \times E_{663}$$

$$\text{Carotenoid (mg mL}^{-1}\text{)} = 4.2 \times E_{452.5} - [(0.0264 \times \text{Chl. a}) + (0.0426 \times \text{Chl. b})]$$

$$\text{Photosynthetic pigment (mg/g FW)} = (C - V/1000 - W)$$

Where E = absorbance, C = conc. of pigment, V = acetone vol. (mL), and W = wt. of sample (g).

2.8.4. Anatomical Structures

At 80 days in the second season, safflower samples represented by the main stems and leaves (fifth internode) were collected from different treatments. Specimens were killed and fixed for 4 days in formalin-aceto-alcohol (10 mL formalin + 5 mL glacial acetic acid + 85 mL 70% ethyl alcohol). Fixed samples were washed in 50% ethyl alcohol, dehydrated in regular series butyl alcohol and embedded in paraffin wax at a melting point of 56°C. Specimens were cross-sectioned to a thickness of 20 μm using a rotary microtome, stained twice with erythrosine crystal violet, cleared in xylene and mounted in Canada balsam (Nassar et al., 2011). The sections were examined using a light microscope and a micrometer eyepiece.

2.9. Data Analyses

The experiments were repeated twice to confirm the results. The data presented were statistically analyzed based on the procedure outlined by Snedecor and Cochran (1980). Means of treatments were compared by least significant difference (LSD) with a probability of 5%. Results were presented as means ± standard deviations (SD).

3. RESULTS

3.1. Observation of Safflower Powdery Mildew (SPM)

In the 2021/2022 season, typical symptoms of powdery mildew were observed on safflower in Beni-Suef governorate, Egypt. The disease mainly attacks leaves and stems, and infection may extend to inflorescences (Figure 1). Initial symptoms appear as scattered, superficial white mycelium forming small, circular or irregular colonies, especially in the lower parts of the plant. As the disease progresses, entire parts of the plant covered with a white, talcum powder-like mycelial mass. Affected leaves become chlorotic, turn blackish-brown and fall.



Figure 1. Typical symptoms of powdery mildew, observed under natural infection conditions on (A) stems; (B) leaves; and (C) inflorescences of safflower compared to (D) healthy plant.

3.2. Survey of Safflower Powdery Mildew

Data presented in Table 4 show that SPM was widespread in the areas surveyed. Initial symptoms appeared in late January in Qena, on February 1st in Beni-Suef, and mid - February in Fayoum. In addition, the severity of

the disease varied between these regions, as it was severe in Qena, followed by Beni-Suef, and less severe in Fayoum, recording 68.3, 57.1, and 54.7%, respectively, for disease incidence, and 47, 42.2, and 36.1%, respectively, for disease severity.

Table 4. Survey of the time of initial symptoms and the incidence and severity of powdery mildew on safflower in three Egyptian governorates during the 2021/2022 season.

Location	Time of Initial Symptoms	Average Incidence %	Disease Average Disease Severity %
Beni-Suef govern.	First February	57.1 ± 2.17 ^b	42.2 ± 1.21 ^b
Fayoum govern.	Mid February	54.7 ± 2.75 ^b	36.1 ± 0.95 ^c
Qena govern.	Late January	68.3 ± 2.23 ^a	47.0 ± 1.82 ^a

Data represent the mean of three replicates ± standard deviation (SD). Different letters indicate a significant difference between means (at $p \leq 0.05$) by Duncan's multiple range test.

3.3. Morphological Characteristics of *Golovinomyces cichoracearum*

As shown in Figure 2 and Table 5, the mycelium was amphigenous, composed of superficial hyphae and had well-developed multilobed appressoria. Conidiophores were erect, straight to slightly curved and consisted of foot cells, followed by straight cells ending in a chain of conidia. Its dimensions ranged between 41-63 (52) µm in length and 9-12 (10.5) µm in width, and the height of conidiophore with conidia ranged between 183-197 µm. Foot cells were cylindrical in shape, adjacent to 2-3 short cells and ranged between 21-39 (30) µm in length and 7.5-9 (8.25) µm in width. Conidia were hyaline, oval to barrel in shape and carried in a long chain on the conidiophore (5-6 conidia per chain). Its dimensions ranged between 20-33

(26.5) µm in length and 10-14 (12) µm in width. Germ tubes emerge from the polar ends of the conidia. Chasmothecia was not observed. Based on these morphological features, the fungus of SPM was identified as *G. cichoracearum* (DC.) V.P. Heluta (Syn.: *E. cichoracearum* DC ex Merat.).

3.4. Pathogenicity of Powdery Mildew Fungus

Data in Table 6 show that *G. cichoracearum* was pathogenic to safflower plants, recording a disease incidence of 41.7% and disease severity of 19.3%. While the control (non-inoculated) remained symptom-free. The developing disease symptoms and the re-isolated fungus were morphologically completely identical to those first observed.



Figure 2. Light microscopy, showing (A) mycelium; (B,C) conidiophores carrying hyaline conidia in long chains; and (D) conidia of *G. cichoracearum* (DC.) V.P. Heluta (Syn.: *E. cichoracearum* DC ex Merat.), the causal agent of SPM.

Table 5. Dimensions and shapes of conidiophores, conidia, and foot cells of *G. cichoracearum*.

Anamorphic stage types	Length (μm)	Width (μm)	Shape
Conidiophores	41-63	9-12	Erect, straight to slightly curved
Conidia	20-33	10-14	Oval to barrel -shaped
Foot cells	21-39	7.5-9	Cylindrical-shaped

Table 6. Pathogenicity test of *G. cichoracearum* against safflower seedlings (cv. Giza 1), 30 days after inoculation under greenhouse conditions.

Tested Fungus	Disease Incidence %	Disease Severity %
<i>G. cichoracearum</i>	41.7 ± 2.25^a	19.3 ± 0.76^a
Control (non-inoculated)	0.0 ± 0.0^b	0.0 ± 0.0^b

Data represent the mean of three replicates \pm standard deviation (SD). Different letters indicate a significant difference between means (at $p \leq 0.05$) by Duncan's multiple range test.

3.5. *In Vitro*, Antifungal Activity of Ethanolic Propolis Extract, Diluted Honey Solution and Clove EO against Conidial Germination of *G. cichoracearum*

Data in Table 7 show that all mixture or individual treatments significantly decreased the conidial germination of *G. cichoracearum* *in vitro*. Also, germination at 48 h of incubation

was higher than at 24 h. Maximum reduction was achieved by EPE (50%) + CEO (3 mL/L), followed by EPE (50%) + DHS (50 g/L) and DHS (50 g/L) + CEO (3 mL/L) as follows: 90, 87.3, and 80.1%, respectively. While the lowest reduction (58.6%) was recorded by DHS (50 g/L). Micronized sulfur (2.5 g/L) was the most superior in reducing germination compared to other treatments (92.4%).

Table 7. Effect of treatments on conidial germination of *G. cichoracearum*, incubated at $25 \pm 2^\circ\text{C}$ and incubation periods of 24 and 48 h.

Treatments	Conidial Germination %			* Reduction %
	Incubation Period (hour)		Mean	
	24	48		
EPE (50%)	6.3 ± 0.26^d	10.7 ± 0.44^c	8.5	74.3
DHS (50 g/L)	12.1 ± 0.17^b	15.3 ± 0.98^b	13.7	58.6
CEO (3 mL/L)	7.3 ± 0.20^c	12.1 ± 0.53^c	9.7	70.6
EPE (50%) + DHS (50 g/L)	3.1 ± 0.17^f	5.3 ± 0.20^e	4.2	87.3
EPE (50%) + CEO (3 mL/L)	3.0 ± 0.17^f	3.7 ± 0.44^{ef}	3.3	90.0
DHS (50 g/L) + CEO (3 mL/L)	5.2 ± 0.36^e	8.1 ± 0.26^d	6.6	80.1
Micronized sulfur (2.5 g/L)	2.3 ± 0.17^f	2.7 ± 0.20^f	2.5	92.4
Control (water only)	30.3 ± 1.39^a	36.0 ± 2.65^a	33.1	—

Data represent the mean of three replicates \pm standard deviation (SD). Different letters indicate a significant difference between means (at $p \leq 0.05$) by Duncan's multiple range test. * Reduction was calculated based on control value.

3.6. Effect of Ethanolic Propolis Extract, Diluted Honey Solution and Clove EO, Individually or in Mixtures on SPM in the Greenhouse

Data in Table 8 reveal that all treatments used in the greenhouse significantly reduced SPM severity and AUDPC compared to control. In addition, mixture treatments were more effective in reducing disease than the individual treatments. The lowest disease severity was

recorded by micronized sulfur, followed by EPE (50%) + CEO (3 mL/L), EPE (50%) + DHS (50 g/L), and DHS (50 g/L) + CEO (3 mL/L) as follows: 7.3, 8.1, 10.7 and 13.1%, respectively. While DHS (50 g/L) recorded the highest disease severity (21.7%). Similarly, the lowest AUDPC values (142.1 and 153.4) were recorded by micronized sulfur and EPE (50%) + CEO (3 mL/L), respectively compared to 618.2 in control.

Table 8. Effect of treatments on the severity and AUDPC of SPM under greenhouse conditions.

Treatments	Average Disease Severity %	AUDPC
EPE (50%)	15.1 ± 0.35 ^d	251.6 ± 7.73 ^d
DHS (50 g/L)	21.7 ± 1.74 ^b	342.8 ± 3.22 ^b
CEO (3 mL/L)	18.3 ± 0.62 ^c	296.2 ± 6.06 ^c
EPE (50%) + DHS (50 g/L)	10.7 ± 0.79 ^f	189.5 ± 10.04 ^f
EPE (50%) + CEO (3 mL/L)	8.1 ± 0.52 ^g	153.4 ± 8.97 ^g
DHS (50 g/L) + CEO (3 mL/L)	13.1 ± 0.53 ^e	223.4 ± 8.81 ^e
Micronized sulfur (2.5 g/L)	7.3 ± 0.26 ^g	142.1 ± 8.41 ^g
Control (water only)	41.3 ± 1.39 ^a	618.2 ± 6.38 ^a

Data represent the mean of three replicates ± standard deviation (SD). Different letters indicate a significant difference between means (at $p \leq 0.05$) by Duncan's multiple range test. AUDPC = area under the disease progress curve.

3.7. Effect of Ethanolic Propolis Extract, Diluted Honey Solution and Clove EO, Individually or in Mixtures on SPM in the Field

Data presented in Table 9 show that all tested treatments significantly reduced the severity of SPM during the 2022/2023 and 2023/2024 seasons compared to control. Also, mixture treatments were more effective in

reducing disease than the individual treatments. The highest disease reduction was achieved by micronized sulfur, followed by EPE (50%) + CEO (3 mL/L), EPE (50%) + DHS (50 g/L), and DHS (50 g/L) + CEO (3 mL/L) as follows: 82.9, 77.5, 72.8, and 67.2 %, respectively. Intermediate values (61.7 and 59.2%) were recorded by EPE (50%) and CEO (3 mL/L), respectively. While DHS (50 g/L) ranked lowest (47.6%).

Table 9. Effect of treatments on the severity of safflower powdery mildew in the field.

Treatments	Average Disease Severity %			* Reduction
	2022/2023	2023/2024	Mean	%
EPE (50%)	16.3 ± 0.75 ^c	14.7 ± 1.91 ^c	15.5	61.7
DHS (50 g/L)	22.1 ± 1.73 ^b	20.3 ± 2.00 ^b	21.2	47.6
CEO (3 mL/L)	18.1 ± 0.79 ^c	15.0 ± 1.14 ^c	16.5	59.2
EPE (50%) + DHS (50 g/L)	11.7 ± 0.44 ^e	10.3 ± 0.26 ^d	11.0	72.8
EPE (50%) + CEO (3 mL/L)	9.3 ± 0.89 ^f	8.9 ± 0.46 ^{de}	9.1	77.5
DHS (50 g/L) + CEO (3 mL/L)	13.7 ± 0.70 ^d	13.0 ± 0.56 ^c	13.3	67.2
Micronized sulfur (2.5 g/L)	7.1 ± 0.40 ^g	6.7 ± 0.26 ^e	6.9	82.9
Control (water only)	42.0 ± 1.95 ^a	39.1 ± 2.52 ^a	40.5	—

Data represent the mean of three replicates ± standard deviation (SD). Different letters indicate a significant difference between means (at $p \leq 0.05$) by Duncan's multiple range test. * Reduction was calculated based on control value.

3.8. Growth and Yield Characteristics

As offered in Figure 3, all treatments significantly improved safflower growth

features, including plant height and number of leaves. The mixture treatments had a better effect than the individual treatments.

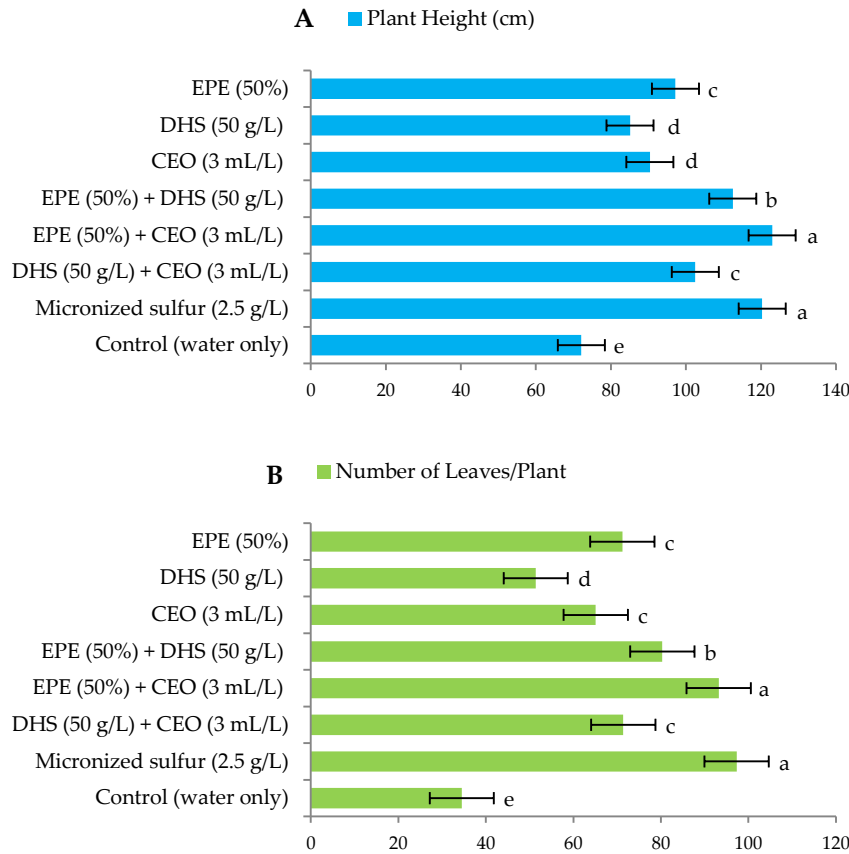


Figure 3. Effect of treatments on safflower growth parameters, including (A) plant height and (B) number of leaves/plant. Data represent the mean of two experiments repeated over the 2022/2023 and 2023/2024 seasons. Vertical bars represent are the mean of three replicates \pm standard deviation (SD). Different letters indicate a significant difference between means (at $p \leq 0.05$) by Duncan's multiple range test.

In this regard, plants treated with EPE (50%) + CEO (3 mL/L), followed by micronized sulfur, EPE (50%) + DHS (50 g/L), and DHS (50 g/L) + CEO (3 mL/L) recorded the highest values of plant height as follows: 123, 120.3, 112.5, and 102.5 cm, respectively. While plants treated with DHS (50 g/L) noted the lowest value (85.1 cm) (Figure 3A). Also, the highest number of leaves/plant was noted by micronized sulfur, followed by EPE (50%) + CEO (3 mL/L) and EPE (50%) + DHS (50 g/L) as follows: 97.3, 93.2, and 80.3, respectively. While DHS (50 g/L) had the lowest value (51.4) (Figure 3B).

As presented in Figure 4, all treatments significantly improved safflower yield, such as number of heads, number of seeds, and weight of 100 seeds in both seasons. The mixture treatments had a better effect than the individual treatments. All plants treated with EPE (50%) +

CEO (3 mL/L), followed by micronized sulfur and EPE (50%) + DHS (50 g/L) recorded the highest values of number of heads/plant as follows: 14.3, 12.1, and 11.3, respectively. While plants treated with DHS (50 g/L) recorded the lowest value (7.1) (Figure 4A). Also, the highest number of seeds/plant was recorded by EPE (50%) + CEO (3 mL/L), followed by micronized sulfur, EPE (50%) + DHS (50 g/L) and DHS (50 g/L) + CEO (3 mL/L) as follows: 38.1, 35.2, 31.7, and 27.4, respectively, while DHS (50 g/L) had the lowest value (17.4) (Figure 4B). Moreover, the highest value of weight of 100 seeds (8.1 g) was recorded by EPE (50%) + CEO (3 mL/L) and micronized sulfur, followed by EPE (50%) + DHS (50 g/L) (7.8 g). While DHS (50 g/L) recorded the lowest value (6.2 g) (Figure 4C).

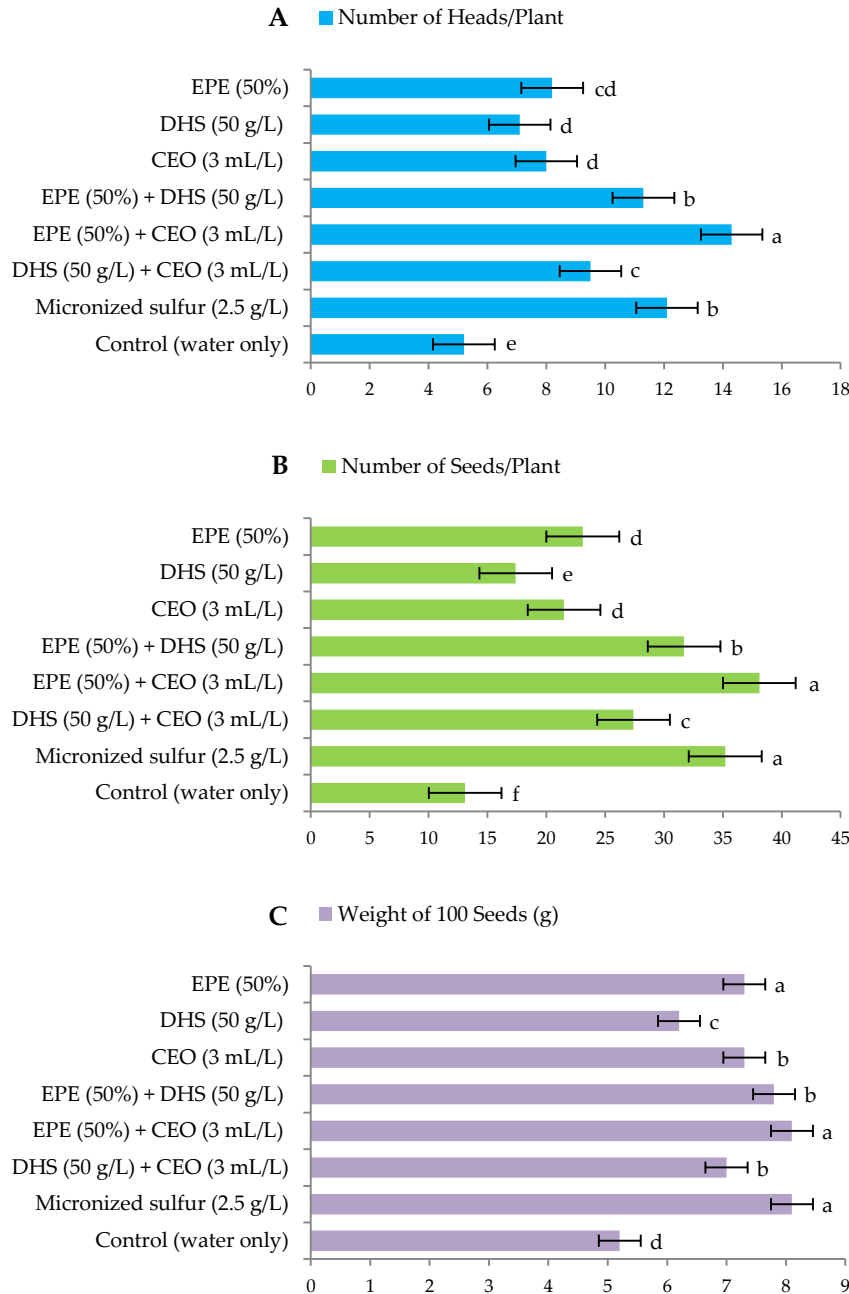


Figure 4. Effect of treatments on safflower yield parameters, including (A) number of heads/plant; (B) number of seeds/plant; and (C) weight of 100 seeds (g). Data represent the mean of two experiments repeated over the 2022/2023 and 2023/2024 seasons. Vertical bars represent are the mean of three replicates \pm standard deviation (SD). Different letters indicate a significant difference between means (at $p \leq 0.05$) by Duncan's multiple range test.

3.9. Photosynthetic Pigments

All treatments significantly increased chlorophyll a, chlorophyll b, and carotenoids (Figure 5). The highest values of these pigments were found in plants treated with EPE (50%) + CEO (3 mL/L), followed by micronized sulfur (2.5 g/L) and EPE (50%) + DHS (50 g/L) as

follows: 9.2, 7.2, and 7.1 mg/g FW, respectively in chlorophyll a, 4.5, 4.1, and 3.3 mg/g FW, respectively in chlorophyll b, and 0.9, 0.81, and 0.79 mg/g FW, respectively in carotenoids. Conversely, plants treated with DHS (50 g/L) recorded the lowest values of 5.3, 2.1, and 0.51 mg/g FW for chl. a, chl. b, and carotenoids, respectively.

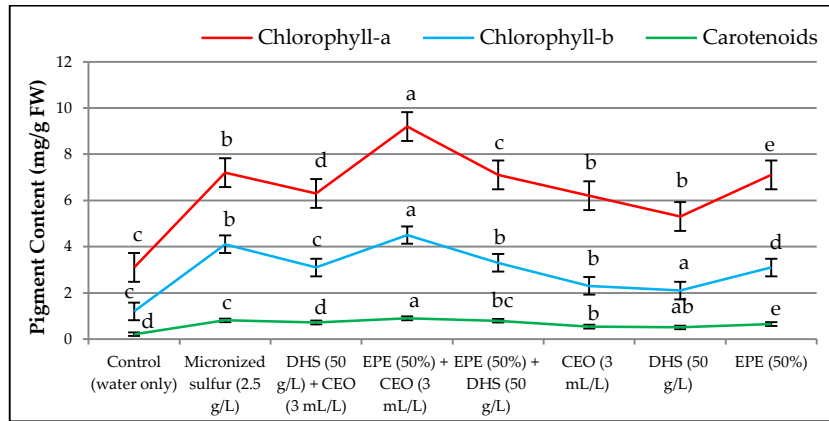


Figure 5. Effect of treatments on chlorophyll (a, b) and carotenoids. Data represent the mean of two experiments repeated over the 2022/2023 and 2023/2024 seasons. Vertical bars represent the mean of three replicates \pm standard deviation (SD). Different letters indicate a significant difference between means (at $p \leq 0.05$) by Duncan's multiple range test.

3.10. Anatomical Characteristics

3.10.1. Stem Structure

Data offered in Table 10 and Figure 6 show that all treatments resulted in obvious improvement in the anatomical features of safflower stems compared to untreated plants. In particular, treatments EPE (50%) + CEO (3 mL/L) and micronized sulfur (2.5 g/L) outperformed other treatments. In this regard, plants treated with EPE (50%) + CEO (3 mL/L) gave the best results for stem width and pith width as follows: 5958 and 3383 μm ,

respectively, compared to 2992 and 1558 μm , respectively in control. In addition, plants treated with micronized sulfur (2.5 g/L) gave the best results for pith length and VB diameter as follows: 4192 and 786 μm , respectively, compared to 2100 and 511 μm , respectively in control. While the best results for stem length and XV diameter were recorded by treatment EPE (50%) + DHS (50 g/L) and EPE (50%) as follows: 7450 and 47.9 μm , respectively, compared to 4250 and 25.9 μm , respectively in control.

Table 10. Effect of treatments on the stem structure of safflower grown under field conditions.

T.	Stem Dimensions		Pith Dimensions		VB Diameter (μm)	XV Diameter (μm)
	Length (μm)	Width (μm)	Length (μm)	Width (μm)		
1	6058 \pm 63 ^c	4542 \pm 72 ^d	3500 \pm 95 ^c	2500 \pm 95 ^d	746 \pm 11.3 ^b	47.9 \pm 2.4 ^a
2	6058 \pm 63 ^c	3558 \pm 63 ^f	3558 \pm 63 ^c	2250 \pm 20 ^e	602 \pm 10.8 ^e	28.6 \pm 2.1 ^d
3	5625 \pm 125 ^d	3842 \pm 80 ^e	2958 \pm 72 ^d	2133 \pm 38 ^e	604 \pm 05.9 ^e	38.6 \pm 1.1 ^c
4	7450 \pm 66 ^a	4500 \pm 50 ^d	4050 \pm 66 ^b	2500 \pm 50 ^d	661 \pm 15.1 ^d	41.2 \pm 0.9 ^{bc}
5	6875 \pm 125 ^b	5958 \pm 72 ^a	4000 \pm 20 ^b	3383 \pm 63 ^a	702 \pm 08.1 ^c	46.4 \pm 1.0 ^a
6	6792 \pm 72 ^b	5025 \pm 66 ^c	3558 \pm 63 ^c	2808 \pm 63 ^c	646 \pm 17.0 ^d	41.6 \pm 2.1 ^b
7	6750 \pm 125 ^b	5708 \pm 72 ^b	4192 \pm 63 ^a	3083 \pm 72 ^b	786 \pm 14.5 ^a	45.4 \pm 0.4 ^a
8	4250 \pm 125 ^e	2992 \pm 63 ^g	2100 \pm 43 ^e	1558 \pm 63 ^f	511 \pm 17.1 ^f	25.9 \pm 2.3 ^d

T1 = EPE (50%), T2 = DHS (50 g/L), T3 = CEO (3 mL/L), T4 = EPE (50%) + DHS (50 g/L), T5 = EPE (50%) + CEO (3 mL/L), T6 = DHS (50 g/L) + CEO (3 mL/L), T7 = micronized sulfur (2.5 g/L), and T8 = control. VB = vascular bundle and XV = xylem vessel. Data represent the mean of three replicates \pm standard deviation (SD). Different letters indicate a significant difference between means (at $p \leq 0.05$) by Duncan's multiple range test.

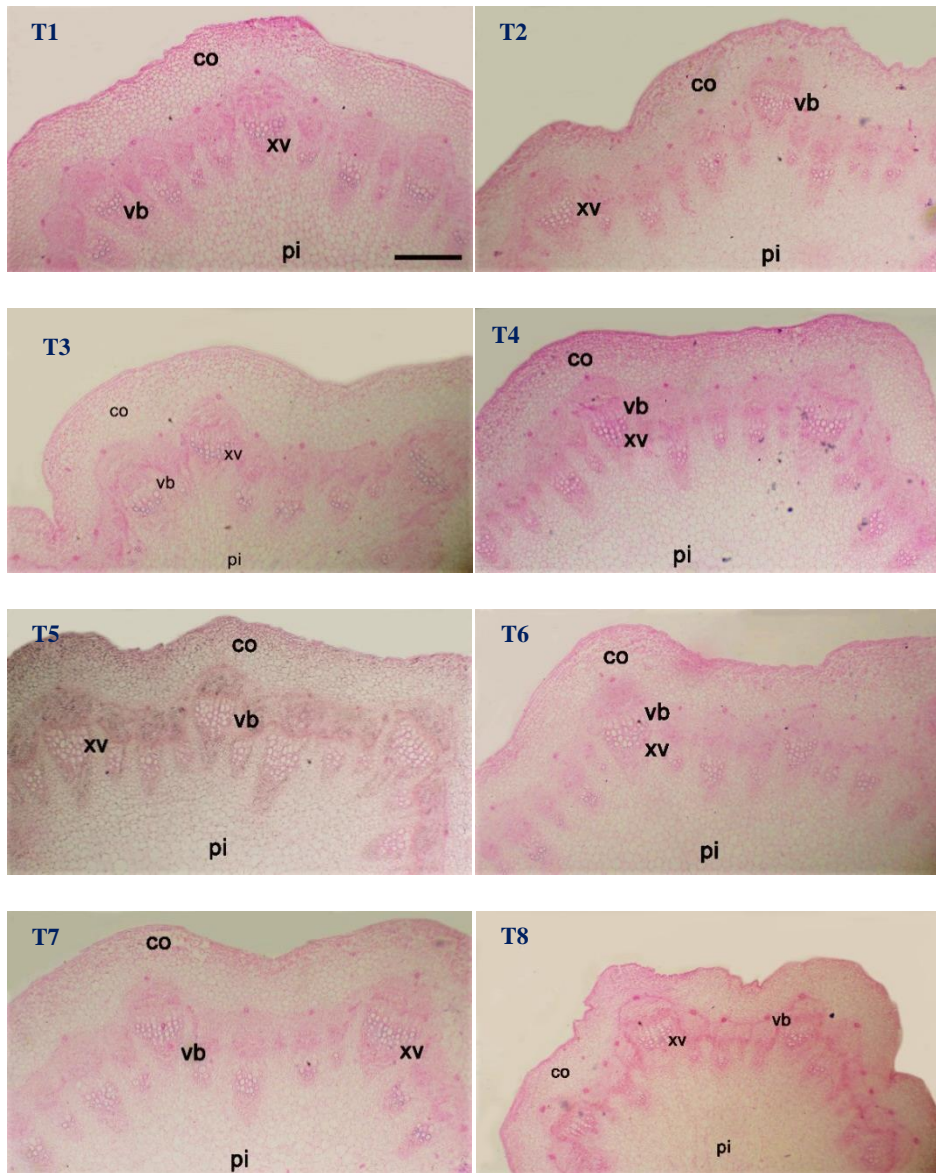


Figure 6. Cross transections in safflower stems grown under the effect of treatments: T1 = EPE (50%), T2 = DHS (50 g/L), T3 = CEO (3 mL/L), T4 = EPE (50%) + DHS (50 g/L), T5 = EPE (50%) + CEO (3 mL/L), T6 = DHS (50 g/L) + CEO (3 mL/L), T7 = micronized sulfur (2.5 g/L), and T8 = control. Pi = pith, vb = vascular bundle, xv = xylem vessels, co = cortex, and Bar = 500 μ m.

3.10.2. Leaf Structure

Data offered in Table 11 and Figure 7 show that all treatments significantly improved the anatomical features of safflower leaves compared to untreated plants. In particular, treatment with micronized sulfur (2.5 g/L), EPE (50%) + CEO (3 mL/L), EPE (50%) + DHS (50 g/L), and DHS (50 g/L) + CEO (3 mL/L) showed better anatomical characteristics than other treatments. In this regard, the best results for thickness of the leaf blade, palisade tissue,

and spongy were obtained by treatment with micronized sulfur (2.5 g/L) as follows: 457, 148, and 234 μ m, respectively compared to 274, 87, and 140 μ m, respectively in control. In addition, the highest values of VB height, VB width, and XV diameter were recorded by treatment with EPE (50%) + CEO (3 mL/L) as follows: 807, 820, and 59.8 μ m, respectively compared to 427, 288, and 37.9 μ m, respectively in control. While the best result for the number of xylem vessels and midvein height were recorded by treatment EPE (50%) + CEO (3 mL/L) and CEO (3 mL/L).

Table 11. Effect of treatments on the leaf structure of safflower grown under field conditions.

T.	LB Thickness (µm)	PT Thickness (µm)	S Thickness (µm)	M Height (µm)
1	311 ± 4.7 ^e	105 ± 5.4 ^c	168 ± 1.7 ^{de}	1737 ± 38.9 ^b
2	324 ± 10.3 ^d	95 ± 8.1 ^{cd}	180 ± 3.4 ^c	1488 ± 37.3 ^d
3	371 ± 6.9 ^b	124 ± 1.1 ^b	177 ± 7.6 ^{cd}	1912 ± 04.7 ^a
4	369 ± 10.3 ^b	123 ± 8.1 ^b	180 ± 3.4 ^c	1657 ± 37.3 ^c
5	342 ± 6.5 ^c	116 ± 5.3 ^b	196 ± 8.0 ^b	1855 ± 08.1 ^a
6	343 ± 3.0 ^c	105 ± 0.0 ^c	166 ± 3.0 ^e	1866 ± 56.9 ^a
7	457 ± 9.7 ^a	148 ± 4.7 ^a	234 ± 6.4 ^a	1786 ± 09.6 ^b
8	274 ± 1.6 ^f	87 ± 5.3 ^d	140 ± 1.2 ^f	1345 ± 38.6 ^e

T.	VB Height (µm)	VB Width (µm)	No. of XV	XV Diameter (µm)
1	748 ± 10.6 ^c	408 ± 13.0 ^d	31.3 ± 1.2 ^c	46.2 ± 1.0 ^d
2	494 ± 12.7 ^e	626 ± 10.2 ^b	26.0 ± 1.0 ^d	44.8 ± 1.7 ^d
3	668 ± 11.0 ^d	432 ± 13.3 ^d	20.0 ± 1.0 ^e	41.3 ± 2.2 ^e
4	754 ± 12.7 ^c	532 ± 10.2 ^c	33.0 ± 1.0 ^{bc}	54.7 ± 1.7 ^{bc}
5	807 ± 8.5 ^a	820 ± 19.9 ^a	36.0 ± 1.0 ^a	59.8 ± 1.6 ^a
6	781 ± 17.1 ^b	554 ± 11.9 ^c	34.0 ± 1.0 ^b	56.9 ± 1.9 ^b
7	780 ± 16.3 ^b	644 ± 18.4 ^b	36.0 ± 1.0 ^a	53.5 ± 1.5 ^c
8	427 ± 10.3 ^f	288 ± 15.6 ^e	17.6 ± 0.6 ^f	37.9 ± 0.4 ^f

T1 = EPE (50%), T2 = DHS (50 g/L), T3 = CEO (3 mL/L), T4 = EPE (50%) + DHS (50 g/L), T5 = EPE (50%) + CEO (3 mL/L), T6 = DHS (50 g/L) + CEO (3 mL/L), T7 = micronized sulfur (2.5 g/L), and T8 = control. LB = leaf blade, PT = palisade tissue, S = spongy, M = midvein, VB = vascular bundle, and XV = xylem vessels. Data represent the mean of three replicates ± standard deviation (SD). Different letters indicate a significant difference between means (at $p \leq 0.05$) by Duncan's multiple range test.

4. DISCUSSION

Powdery mildew is one of the most common and widespread fungal diseases in the world, infecting the stems, leaves, flowers, and fruits of about 10,000 angiosperm species (Glawe, 2008). In the last 2-3 years, powdery mildew has become a major challenge for safflower cultivation in Egypt. This disease has been previously reported on safflower in many countries of the world, including the United States (Berkenkamp, 1961), California (McCain, 1962), India (Saluja and Bhide, 1963), Karnataka (Siddaramaiah et al., 1981), and Korea (Kwon et al., 2000). Our results reported that based on the examined morphological characteristics of conidiophores, conidia, and foot cells, the causal agent of safflower powdery mildew was identified as the fungus *G. cichoracearum* (DC.) V.P. Heluta (Syn.: *E. cichoracearum* DC ex Merat.). This finding is consistent with that reported by Braun (1999) and Braun and Cook (2012). Several previous reports documented the first observation of *G. cichoracearum* on safflower plants in many countries, such as California, India, and Karnataka (McCain, 1962; Saluja and Bhide,

1963; Siddaramaiah et al., 1981). As reported by Braun (1987), *G. cichoracearum* is widespread worldwide and can attack many members of the Asteraceae family, including sunflower, lettuce, zinnia, marigold, artichoke, and chicory (Lebeda, 1985; Blancard et al., 2006; Koike et al., 2007; Baiswar et al., 2008; Severoglu and Ozyigit, 2012). To our best knowledge, this is the first report of safflower powdery mildew in Egypt (Farr and Rossman, 2021). According to our field observations, this disease poses a real threat to the production of this crop, especially those grown under organic farming systems in which chemicals are limited. This paper discusses the use of ethanolic propolis extract, diluted honey solution, and clove EO, as natural, eco-friendly alternatives to synthetic fungicides for controlling safflower powdery mildew.

Our results showed that all mixture or individual treatments significantly reduced conidial germination of *G. cichoracearum* *in vitro*. Maximum reduction was achieved by EPE (50%) + CEO (3 mL/L), followed by EPE (50%) + DHS (50 g/L), and DHS (50 g/L) + CEO (3 mL/L). Numerous reports have proved strong activity of propolis against plant pathogenic

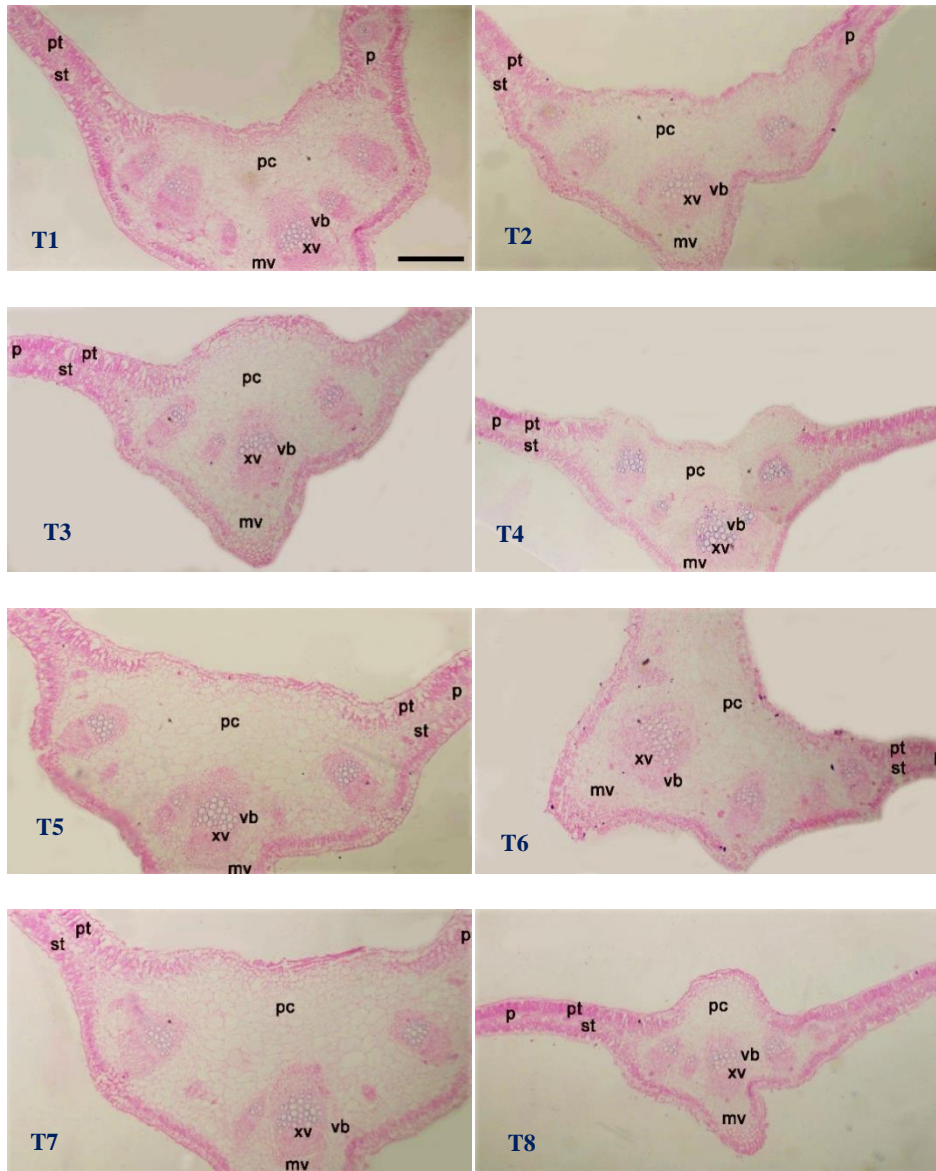


Figure 7. Cross sections in safflower leaves grown under the effect of treatments: T1 = EPE (50%), T2 = DHS (50 g/L), T3 = CEO (3 mL/L), T4 = EPE (50%) + DHS (50 g/L), T5 = EPE (50%) + CEO (3 mL/L), T6 = DHS (50 g/L) + CEO (3 mL/L), T7 = micronized sulfur (2.5 g/L), and T8 = control. St = sponge parenchyma tissue, pt = palisade tissue, pc = parenchyma cells, xv = xylem vessels, vb = vascular bundle of the midvein, mv = midvein, and Bar = 500 μ m.

fungi under *in vitro* conditions. Among these studies is Er (2021), who found that ethanolic propolis extract and water-based propolis had high inhibitory effects of up to 97% against the mycelial growth of *P. ultimum*, *V. dahliae*, *A. brassicicola*, and *F. graminearum*. Similarly, Çakar et al (2022) tested the inhibitory activity of three types of propolis extracts against mycelial growth of *F. solani*. The results showed that the pure extract was the most effective, followed by the ethanol extract, while the DMSO (dimethyl sulfoxide) extract was the

least effective, recording 30, 33, and 58 mm in mycelium diameter, respectively. This antifungal activity of propolis may be due to the presence of high amounts of phenolic compounds such as phenolic acids and flavonoids (Velazquez et al., 2007). Regarding the antimicrobial activity of diluted honey solution, several studies have demonstrated its potential against a number of pathogenic bacteria, including about 60 species of aerobic, anaerobic, Gram-positive, and Gram-negative bacteria. Among these bacteria are *A.*

baumannii, *B. cereus*, *E. aerogenes*, *P. aeruginosa*, *M. luteus*, *S. flexneri*, *S. marcescens*, and *S. aureus* (Hannan et al., 2004; Hegazi et al., 2017). This activity of honey may be due to it containing high amounts of phenolic compounds (Saxena et al., 2010). Although the strong antibacterial activity of diluted honey solution, our knowledge of its antifungal activity is still limited. On the other hand, extensive reports have shown the efficacy of clove EO against fungal pathogens *in vitro*. For example, Ahmed et al. (2023a) establish that clove EO at 1 mL/L completely reduced the mycelium growth of *S. sclerotiorum* and *F. solani*. Similarly, Sharma et al. (2017) found that clove EO was most efficient in reducing mycelium growth and conidial germination of *F. oxysporum* f. sp. *lycopersici*. This activity of clove EO may be due to it containing a high amount of phenolic compounds (eugenol; 75-100%), known for its antimicrobial properties (El-Saber Batiha et al., 2020). Eugenol can denature proteins and interact with phospholipids in the pathogen cell membrane, altering the permeability (Bhuiyan et al., 2010).

Our findings showed that all treatments significantly reduced disease severity and AUDPC in the greenhouse compared to untreated plants, and the mixture treatments were more effective than the individual treatments. Similar results were obtained in field trials, where the most effective treatments were micronized sulfur (2.5 g/L), EPE (50%) + CEO (3 mL/L), EPE (50%) + DHS (50 g/L), and DHS (50 g/L) + CEO (3 mL/L). Several reports have documented the successful use of propolis to control plant diseases under *in vivo* conditions. For example, treatment of bean, grapevine, cucumber, and strawberry with propolis extract reduced fungal diseases caused by *B. cinerea*, *P. fuliginea*, *R. solani*, *P. digitatum*, and *S. rolfisii* (Abd-El-Kareem et al., 2018). Also, Heck et al. (2015) found that treatment of cucumber with ethanolic propolis extract (8%) significantly reduced powdery mildew severity and AUDPC. Moreover, it was found that coating banana fruits with a mixture of propolis (50%) and paraffin wax had an effective effect against crown rot that was similar to the effect of the fungicide Prochloraz (Sripong et al., 2020). Extensive reports have shown the efficacy of clove EO in controlling plant pathogens. Ahmed

et al. (2023a) reported that application of clove EO as seed soaking (3 mL/L) and foliar spray (1.5 mL/L), significantly reduced root rot and wilt of marigold. Similarly, clove EO (1500 ppm) completely prevented guava from fungal decline (Hamad et al., 2015). Also, Abdel-Kader et al. (2011) found that coating the seeds of bean with clove EO significantly reduced root rot in the pre and post emergence stages. The antimicrobial properties of clove EO may be due to it containing a large amount of phenolic compounds known for their antifungal, antibacterial, and insecticidal activity (El-Saber Batiha et al., 2020). As stated by Draz et al. (2019), plant extracts and essential oils suppress pathogenic fungi by increasing the mortality (fungicidal effect), reversing the growth and development (fungistatic effect) and/or promoting the growth of plant by eliciting host defense reactions. The current study also showed that micronized sulfur achieved the greatest control of the disease compared to other treatments. This action may be a result of interference with cell wall synthesis, increased permeability of cell walls, destruction of the plasma membrane, or inhibition of ergosterol synthesis, which is necessary for cell wall synthesis (Ahmed et al., 2023b).

In the current study, disease reduction due to the effect of treatments was expressed through improved growth, yield, and anatomical characteristics of the plant. The mixture treatments had a better impact than the individual treatments. The most effective treatments were EPE (50%) + CEO (3 mL/L), micronized sulfur (2.5 g/L), EPE (50%) + DHS (50 g/L), and DHS (50 g/L) + CEO (3 mL/L). In fact, natural-based biostimulants such as propolis and diluted honey extracts play a vital role in stimulating the growth and productivity of crops, in addition to enhancing the plant's ability to decrease the influences of various stresses, indirectly by obstructing pathogenic conditions and directly by improving plant growth (Rouphael and Colla, 2018; Abdel-Wahed et al., 2024). The activity of biostimulants to promote plant growth is due to their containment of molecules, such as osmoprotectants, antioxidants, mineral nutrients, vitamins, amino acids, enzymes, and plant hormones. These molecules activate some physiological and biochemical changes in the

plant. It also increases water and nutrient uptake, and reduces excess ROS, which are a source of oxidative stress caused by different stresses (Semida et al., 2019). As reported by Teklic'et al. (2020), diluted honey extract as a plant biostimulant can increase salt stress tolerance in onion and increase biomass production, bulb productivity, and photosynthetic pigment content, as well as stimulate non-enzymatic and enzymatic antioxidants, water content, and membrane integrity. Our findings showed that all treatments significantly increased the content of chlorophylls and carotenoids in both seasons. The maximum values of these pigments were found in plants treated with EPE (50%) + CEO (3 mL/L), followed by micronized sulfur (2.5 g/L) and EPE (50%) + DHS (50 g/L). A similar finding was also achieved by Ahmed et al. (2023a), who reported that treatment of marigold with clove EO significantly increased the content of total chlorophyll and carotenoids. The activity of clove EO, propolis, and honey extracts in increasing photosynthetic pigments may be attributed to their ability to stimulate some hormones and uptake mineral elements such as manganese and iron necessary for the biosynthesis of these pigments (Ben Maachia et al., 2013). In addition to its ability to stimulate the formation of pyridoxal enzymes necessary for the synthesis of beta-aminolevulinic compound, which plays a vital role in chlorophyll biosynthesis (Adil et al., 2017).

5. CONCLUSION

Powdery mildew caused by *G. cichoracearum* (DC.) V.P. Heluta has become one of the serious safflower diseases recently observed in Egypt. Treatment with EPE, DHS, and CEO, individually or in mixtures, has achieved positive results in disease reduction *in vitro* and *in vivo* conditions. All treatments significantly reduced conidial germination of *G. cichoracearum in vitro*, as well as SPM severity and AUDPC *in vivo*. The mixture treatments were more effective than the individual treatments. The most effective treatments were micronized sulfur, EPE (50%) + CEO (3 mL/L), EPE (50%) + DHS (50 g/L), and DHS (50 g/L) + CEO (3 mL/L). The reduction in SPM due to the effect of treatments was expressed via improving growth, yield, photosynthetic

pigments, and anatomical characteristics of the plant.

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المخلص العربي

ملاحظة البياض الدقيقي على القرطم في مصر، ومقاومته باستخدام البروبوليس والعسل المخفف والزيت العطري للقرنفل

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أصبح البياض الدقيقي في الآونة الأخيرة مشكلة خطيرة تهدد العنصر في مناطق زراعته في مصر. يصيب المرض بصفة أساسية الاوراق والسيقان، وقد تمتد الاصابة الى النورات مسببة خسائر فادحة. لوحظ ان المرض كان واسع الانتشار في المناطق التي شملها الحصر، وعلى الرغم من ذلك اختلفت شدته بين هذه الاماكن، حيث كان شديداً في محافظة قنا، تلاها بني سويف، في حين كان اقل حدة في الفيوم. بناءً على الخصائص المورفولوجية للطور اللاجنسي، تم تعريف المسبب المرضي على انه الفطر *Golovinomyces cichoracearum*. في هذه الدراسة، قمنا باختبار نشاط المستخلص الايثانولي للبروبوليس، ومحلول العسل المخفف، والزيت العطري للقرنفل، بصورة فردية او في مخاليط ضد المرض في المعمل والحقل. أدت جميع المعالجات إلى انخفاض ملحوظ في الانبات الكونيدي للفطر في المعمل. تم تحقيق الحد الأقصى من التخفيض بواسطة المستخلص الايثانولي للبروبوليس (٥٠%) + زيت القرنفل (٣ مل/لتر)، تلاه المستخلص الايثانولي للبروبوليس (٥٠%) + محلول العسل المخفف (٥٠ جم/لتر)، ثم محلول العسل المخفف (٥٠ جم/لتر) + زيت القرنفل (٣ مل/لتر) على النحو التالي: ٩٠، ٨٧، ٣، ٨٠، ١، % على الترتيب. في حين تم تسجيل اقل انخفاض بواسطة محلول العسل المخفف (٥٠ جم/لتر). أدت جميع المعالجات الى انخفاض كبير في شدة المرض والمنطقة الواقعة تحت تقدم المرض (AUDPC) في الصوبة. وكانت المعالجات الخليطة اكثر فعالية من المعالجات الفردية. وتم الحصول على نتائج مماثلة في الحقل خلال موسمين متتاليين. تم التعبير عن الانخفاض في شدة المرض من خلال تحسين صفات النمو والانتاج واصباغ التمثيل الضوئي والخصائص التشريحية للنبات. بشكل عام، أمكن استخدام المستخلص الايثانولي للبروبوليس ومحلول العسل المخفف والزيت العطري للقرنفل بنجاح لمقاومة البياض الدقيقي وتحسين النمو والإنتاجية في القرطم.