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The influence of certain eco-friendly treatments on the barley powdery mildew disease under the impact of climate change

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Abstract

This investigation's goal is to assess the effectiveness of eco-friendly treatments, *i.e.*, biocide Blight stop, three natural oil extracts: black seeds, thyme and eucalyptus oils, as well as fungicide Opus 12.5% Sc in reducing powdery mildew infection on susceptible Egyptian barley varieties. These treatments were carried out under greenhouse and field experiments conducted at Giza Experimental Station, Agricultural Research Centre (ARC), during the 2020/21 and 2021/22 growing seasons. All eco-friendly treatments and fungicide reduced disease severity percentage, area under the disease progress curve (AUDPC) and average coefficient of infection (ACI) compared to the control treatment in the two seasons, in addition to increasing yield, total chlorophyll and carotenoids compared to the control treatment. Spraying fungicide Opus provided the most effective treatments, followed by biocide-Blight stop. On the other hand, thyme oil was recorded as having the least effectiveness in comparison to the control treatment during both seasons.

Keywords: barley, powdery mildew, biological control, oil extracts, inducers, fungicide.

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1. Introduction

In Egypt, barley is the principal crop that is farmed on a large scale in the North Coastal Province as well as in the recently reclaimed regions with saline soils and a lack of pure water. One of the most major cereal crops worldwide is barley (*Hordeum vulgare* L.). It is used for both human and animal nutrition, according to Malcolmson et al. (2005). The most harmful barley disease, powdery mildew, is caused by the biotrophic fungus *Blumeria graminis* f. sp. *hordei* (Abdullaev et al., 2021; Tratwal and Bocianowski, 2014). The production loss caused by powdery mildew can exceed 30% in moderately temperate and humid climates, with an average loss of 5–10% (Agostinetto et al., 2014). Treatments of barley powdery mildew with fungicides are typically used to control plant diseases (Hafez & El-Baghdady, 2013). However, the emergence of fungicide-resistant pathogenic strains and their detrimental impacts on human health and the environment often make the use of fungicides ineffective in the long term (Bourras et al., 2018). The general idea is to utilize the techniques that are greatest and most environmentally friendly during a certain stage of the farmed plant's development (Newton et al., 2010). The cultivation of modern cultivars in many different kinds of combinations and complicated hybridised groups in line with the theory of evolutionary plant cultivation is one of the relatively simple and inexpensive approaches that increases the lengthy viability of genetic resistance (Matyjaszczyk, 2015). The severity of barley powdery mildew (*Blumeria graminis* f.sp. *hordei*) was dramatically reduced when barley leaves were treated with 0.5% black seed oil (BSO), rapeseed oil (R oil), and paraffin oil (P oil), from 63.4% (comparison group) to 9.4% (BSO), 16% (R oil), and 16.4% (P oil) in comparison with control treatment (Ali & Blunden, 2003). It is clear that the protective action of oils against powdery mildew is mostly caused by inhibition of conidia germination and reduction of the growth of pathogenic fungi, with very little activation of host defence systems according to thymoquinone, the primary ingredient in both the fixed oil and the essential oil (Ali & Blunden, 2003). Therefore, it is crucial to pay greater focus to BSO and other oils that are effective against the microorganisms that cause powdery mildew as safe and adequate alternative control measures for the production of healthy and organic foods (Hafez et al., 2014). The treatment of pepper plants with Blight stop at the rate of 1 l:50 l water recorded the highest percentage of decrease in disease incidence and severity of pepper plants, and also recorded the highest increase in vegetative growth, yield, fruit quality such and total chlorophyll during the two growing seasons 2019/20 and 2020/21 compared with control treatment (Ahmed et al., 2021). It is possible to utilize non-traditional biological substances and *Trichoderma asperilium* 34, a biological commercial product, as an alternative to fungicides for managing the symptoms of the barley's net blotch disease. Additionally, the treatments were successful in that they considerably boosted the yield characteristics compared to the control (Hafez et al., 2019). The goal of this study is to investigate the impact of some environmentally friendly materials, such as plant extract oils and biological control, in order to decrease the use of chemical fungicides for controlling barley powdery mildew, produce barley grains with high quality and quantity without any toxicity at the food chain, and maintain sustainable development over the long term.

2. Materials and methods

2.1 Barley genotypes

Barley genotypes, whose names and pedigrees are listed in Table (1), were kindly provided by the Barley Research Department of the Field Crops Research Institute, Agricultural Research Centre in Egypt. These genotypes were used in both greenhouse and field studies.

Table 1: Name and pedigree of two barley genotypes employed in greenhouse and field experiments during two seasons 2020/21 and 2021/2022.

Cultivar Name	Pedigree
Giza 2000	C .C 89/3/Alanda/Hamra//Alanda-01
Giza 123	Giza 117/FAO 86

2.2 Compounds used to treat powdery mildew on barley plants

In this study, Blight-stop as a biocide, three natural oil extracts: black seeds, thyme and eucalyptus oils, as well as Opus as a chemical pesticide were applied on barley plants to study their effectiveness against barley mildew disease caused by *Blumeria graminis* f.sp. *hordei* throughout the trial seasons of 2020/2021 and 2021/2022, as indicated in Table (2).

Table 2: Technical data sheet of compounds used to treat powdery mildew on barley plants during two successive seasons, 2020/2021 and 2021/2022.

Trade name	Active ingredient	Recommended dose	Source
Opus	12.5% Sc Epoxiconazole	75 ml/100 l	BASF Egypt for Agricultural Solutions, New Cairo, Egypt
Blight Stop	*Trichoderma harzianum* 30×10^6 spore/ml	1 l/100 l	Biological Control Production Unit Central Laboratory of Organic Agriculture, CLOA, ARC, Egypt
Eucalyptus oil	Monoterpens, Sesquieterpenes and Aromatic phenols	20 ml/l	Harraz for Food Industry & Natural Products
Thyme oil	40.5% Thymol, 23.6% P-cymen, 3.2% Carvacrol, 5.4% Inalool, 2.6% B-caryphllene and Terpinen	20 ml/l	Harraz for Food Industry & Natural Products
Black seed oil	Thymoquinone (21.01%), o-Cymene (18.23%), and β-Thujene (17.22%)	20 ml/l	Harraz for Food Industry & Natural Products

2.3 Greenhouse experiments

2.3.1 Samples of diseases and barley powdery mildew isolates

According to the procedures described by Xu et al. (2014), diseased leaves of barley were taken from the disease nurseries' spreading lines, and a single colony of *Blumeria graminis* f.sp. *hordei* (Bgh) isolate was produced and preserved on seedlings of the barley variety in test tubes with a diameter of 5 cm and sterile soil.

2.3.2 Spore processing

Conidia of each isolate from the aforementioned seedlings were placed onto the barley seedlings of Giza 123 and Giza 2000, which were grown in pots with 400 mL of sterilised soil, and then incubated in a growth chamber at 20±2ºC with continuous lighting to multiply the Bgh isolates. Five layers of cheesecloth were placed on the surface of a glass cylinder with a 10 cm diameter to completely enclose the seedlings and avoid cross-contamination. After 3-5 days of white mycelia's emergence, leaf segments of 5 cm in length from the inoculated leaves were cut off, placed upside-down on 1% agar plates, and cultivated at 18±2ºC under a 16/8 h (light/dark) lighting regime. The conidia were collected onto tissue paper in the laminar flow after 5-7 days of incubation and put into 2.0 ml centrifuge tubes. The pathogenicity assay was conducted using fresh conidia (Wang et al., 2023).

2.3.3 Typing in virulence

In the climate-controlled greenhouse of the Barley Diseases Research Department of the Field Crops Research Institute, Agricultural Research Centre, Egypt, barley grains of the varieties Giza 123 and Giza 2000 were grown in clay pots with a diameter of 30 cm for eight days. The most prevalent races of *Blumeria*

graminis f.sp. *hordei* were artificially inoculated into each pot at the 2-leaf stage by carefully shaking the sporulation leaf segments while the plants were grown in a greenhouse at a temperature of 20°C (Nair & Ellingboe, 1962). Twenty-four hours after inoculation, leaves were sprayed with the recommended doses of the aforementioned Blight-stop as a biocide, three natural oil extracts: black seeds, thyme and eucalyptus oils, as well as Opus as a chemical pesticide. Plants were strictly sprayed with distilled water only as a control treatment. Three replicates of each treatment were used in the experiment, which was conducted using a completely randomized block design. In addition, cultural practices and irrigation were applied. According to Jensen et al. (1992), the infection types (ITs) of each barley variety collected from different Bgh isolates were graded on a scale of 0 to 4, and responses for resistance/susceptibility were determined as follows: 0-2 for resistant (R) and 3-4 for susceptible (S). Incubation period (IP) was calculated as the number of days between the inoculation and the first sign of the disease's initial symptoms or signs, such as spots. (Holliday, 2001). Latent period was calculated as the number of days between the day of inoculation and the day when 50% of the conidia emerged and damaged the leaf epidermal (Andrres, 1982). According to the equation which described by Ahmed et al. (2021), the disease severity was determined by randomly analysing the leaves from each treatment., using a 0 – 4 scale, where 0=no disease; 1= 1–10% leaf area affected; 2= 11–25% leaf area affected, 3= 26– 50% leaf area affected and 4≥50% leaf area affected. The formula was used to determine the percentage disease severity index.:

$$\text{DSI} = \frac{\sum (n \times v)}{Z \times N} \times 100$$

Where: DSI= Disease severity index, n = Number of leaves in each category, v = Numerical value of each category, Z= Numerical value of highest category and N = Total number of leaves in the sample. The Reduction in disease severity % was calculated according to the following formula:

$$\text{Reduction in disease severity \%} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

2.4 Filed experiments (adult stage)

In order to evaluate the effectiveness of Blight-stop as a biocide, three natural oil extracts: black seeds, thyme and eucalyptus oils, as well as Opus as chemical pesticide treatments against the natural infection with powdery mildew on the susceptible Egyptian barley varieties, field experiments were conducted at Giza Experimental Station, Agricultural Research Centre (ARC), Egypt during the 2020/2021 and 2021/2022 growing seasons. Seeds Giza 123 and Giza 2000 were studied in randomised complete block design with three replicate plots, each plot was sown in 10.5 m^2 (3 × 3.5 m long), with 20 cm between rows. In accordance with the Ministry of Agriculture's guidelines, all traditional cultural practises were implemented at the appropriate time. All foliar spray treatments mentioned above with recommended doses were separately applied twice, the first time at heading stage (70 days after planting), at the start of the infection, and the second time after 10 days. According to, disease severity was recorded.

2.4.1 Disease assessment under field conditions

In each trial, ten plants at heading stage from each treatment were assessed visually on a 0–10 scale for the percentage of leaf area covered by powdery mildew (Large, 2007). Disease scores were converted for analysis by Hafez et al. (2014), *i.e.*, 0 = 0 %, 1 = 0–3%, 2 = 3–6%, 3 = 6–12%, 4 = 12–25%, 5 = 25–50%, 6 = 50–

75%, 7 = 75–88%, 8 = 88–94%, 9 = 94–97%, and 10 = 97–100%. Disease severity index (DSI) was calculated using the following formula**:**

$$\text{DSI} = \frac{\sum Ratings\ of\ each\ plant}{10 \times Number\ of\ plants\ rated} \times 100$$

Pathan and Park (2006) estimated the coefficient of infection (CI) by multiplying the severity score by a constant value of infection type. The average coefficient of infection (ACI) was computed by taking the mean CI values for two years (Stubbs et al., 1986). The area under disease progress curve (AUDPC) was calculated using a simple formula adopted by (Pandey *et al*., 1989) as follow:

$$\text{AUDPC} = D\ [½\ (Y_1 + Y_K) + (Y_2 + Y_3 + \ldots\ldots\ldots + Y_{K-1})]$$

Whereas D= days between two consecutive (time intervals), $Y_1 + Y_K$ = sum of the first and last disease scores, $Y_2 + Y_3 + \ldots\ldots\ldots + Y_{K-1}$ = sum of all in between disease scores.

2.4.2 Yield components

All harvested plants at maturity stage during the two growing seasons 2020/21 and 2021/22 at Giza station and plots were recorded in terms of biological yield (kg/plot). Following harvesting, the grain yield (kg/plot) was calculated from the harvested plants' or plots' collected grains. The mean weight of 1000-grain samples collected at random was used to calculate the 1000-grain weight (g). The increase over control in yield component was estimated according to the equation adopted by Ahmed (2013) and Hafez et al. (2014) as follow:

$$\text{Increase over control \%} = \frac{\text{Treatment} - \text{Control}}{\text{Control}} \times 100$$

2.4.3 Estimating the amount of chlorophyll and carotenoids

The technique of Litchenthaler (1987) and Yu et al. (2014) was used to determine the total chlorophyll and carotenoids (mg/g).

2.5 Statistical analysis

The statistical analysis software SAS was used to analyse the data. All multiple analysis was initially evaluated through an analysis of variance (ANOVA) comparison of means, the least significant differences (LSD) AT P = 0.05, and Duncan's multiple range test (Duncan, 1995) was used to determine the results.

3. Results

3.1 Greenhouse studies

3.1.1 Effect of some eco-friendly treatments and fungicide on disease severity

According to data in Table (3), spraying different environmentally friendly treatments, such as Blight-stop as a biocide, three natural oil extracts: black seeds, thyme, and eucalyptus oils, and Opus fungicide, significantly decreased the percentages of disease severity in inoculated barley seedling varieties Giza 123 and Giza 2000 with *Blumeria graminis* f.sp. *hordei* under greenhouse conditions as compared to control treatment. The disease severity of powdery mildew on both the Giza 123 and Giza 2000 types is totally controlled by the foliar application of opus fungicide, contrary to other eco-friendly treatments. The biocide Blight stop caused the superior efficacy in reduction (87.36 and 88.24%) followed by black seeds oil (85.06 and 82.35%) of disease severity of powdery mildew on Giza 123 and Giza 2000 barley verities, respectively compared with other oils rather than untreated plants. On the opposite side thyme oil caused the least effect one. Finally, Giza 123 barley variety is higher sensitive for infection with powdery mildew than Giza 2000 during greenhouse conditions.

Table 3: Effect of some eco-friendly treatments and fungicide as foliar spray on disease severity percentage of inoculated barley seedling varieties Giza 123 and Giza 2000 with *Blumeria graminis* f.sp. *hordei* under greenhouse conditions.

Treatments	Concentrations	Barley cultivars			
		Giza 123		Giza 2000	
		Disease severity (%)	Reduction (%)	Disease severity (%)	Reduction (%)
Blight stop	1 l/100 l	11.00	87.36	10.00	88.24
Black seeds oil	20 ml/l	13.00	85.06	15.00	82.35
Eucalyptus oil	20 ml/l	18.00	79.31	20.00	76.47
Thyme oil	20 ml/l	28.00	67.82	30.00	64.71
Opus	0.75 cm^3/l	00.00	100.00	00.00	100.00
Untreated	Only water	87.00	0.00	85.00	0.00
LSD at 5%		2.12		2.11	

3.1.2 Effect of some eco-friendly treatments and fungicide on incubation and latent periods (day) of barley powdery mildew

Powdery mildew infection of two barley varieties Giza 123 and Giza 2000, expressed as incubation period (IP) and latent period (LP) were affected differently after spraying primary leaves of barley seeds with the tested ecofriendly and Optus fungicide treatments compared with untreated plants. Data in Table (4) revealed that, the foliar spraying of barley seedlings with Blight-stop compounds as a biocide, three natural oil extracts (black seeds, thyme, and eucalyptus oils), and the fungicide Opus showed their efficacy in lengthening the incubation period (IP) and the latent period (LP), which increased their effectiveness in decreasing the severity of infection with the barley powdery mildew, *Blumeria graminis* f.sp. *hordei*, as seen at the previous Table (3), compared to the control treatment. In light of this, the fungicide Opus demonstrated a significant increase in the lengthening of the incubation period (IP) of 76.68% and the latent period (LP) of 40.00% for the barley powdery mildew *Blumeria graminis* f.sp. *hordei* when compared to other natural oil extracts. Thyme oil extract, on the other hand, showed the least significant increase one.

Table 4: Effect of different eco-friendly treatments and fungicide on incubation and latent periods (day) of inoculated barley seedling varieties Giza 123 and Giza 2000 with *Blumeria graminis* f.sp. *hordei* under greenhouse conditions.

Barley varieties	Treatments	Concentrations	Disease severity (%)			
			Incubation period (day)	Increase (%)	Latent periods (day)	Increase (%)
Giza 123	Blight stop	1 l/100 l	9.75	72.26	11.25	36.36
	Black seeds oil	20 ml/l	8.44	49.12	10.42	26.30
	Eucalyptus oil	20 ml/l	7.23	27.74	9.25	12.12
	Thyme oil	20 ml/l	6.98	23.32	9.00	9.09
	Opus	0.75 cm^3/l	10.00	76.68	11.55	40.00
Control	Untreated	Only water	5.66	0.00	8.25	0.00
LSD at 5%			0.92		1.18	
Giza 2000	Blight stop	1 l/100 l	9.65	84.87	10.83	35.21
	Black seeds oil	20 ml/l	8.12	55.56	10.02	25.09
	Eucalyptus oil	20 ml/l	7.10	36.02	9.12	13.86
	Thyme oil	20 ml/l	6.21	18.97	8.22	2.62
	Opus	0.75 cm^3/l	10.00	91.57	11.55	44.19
Control	Untreated	Only water	5.22	0.00	8.01	0.00
LSD at 5%			0.91		1.01	

3.2 Filed experiments

3.2.1 The effect of ecofriendly treatment on barley powdery mildew during 2020/21 and 2021/22 growing seasons

This investigation evaluated the efficacy of Blight-stop as a biocide, three natural oil

extracts: black seeds, thyme, and eucalyptus oils, as well as Opus as a chemical pesticide with recommended doses as mentioned above at Table (2) on powdery mildew of Giza 2000 and Giza 123 barley varieties *in vivo* trials during 2020/2021 and 2021/2022 growing seasons. The efficiency of foliar spraying with tested ecofriendly and fungicide treatments were employed to evaluate the barley powdery mildew infection, which was expressed as average percentages of mildewed leaf area (PM disease severity %), area under the disease progress curve (AUDPC), and average coefficient of infection (ACI). The results in Tables (5.a and 5.b) indicated that all environmentally friendly and fungicide treatments are highly effective in reducing the severity of the barley powdery mildew disease, the ACI, and the AUDPC when compared to control treatments on both barley variety, Giza 123 and Giza 2000, during two successive seasons, 2020/2021 and 2021/2022.

Table 5a: Effect of spraying different eco-friendly treatments and fungicide on disease severity percentage, average coefficient of infection (ACI) and area under disease progress curve (AUDPC) of *Blumeria graminis* f.sp. *hordei* on barley varieties Giza 123 and Giza 2000 under field conditions during the 2020/2021 growing season.

Barley varieties	Treatments	Concentrations	Disease severity (%)	Reduction (%)	ACI	Reduction (%)	AUDPC	Reduction (%)
Giza 123	Blight stop	1 l/100 l	10.22	87.74	3.57	95.22	52.50	89.30
	Black seeds oil	20 ml/l	15.60	81.28	4.17	94.41	86.60	82.36
	Eucalyptus oil	20 ml/l	20.45	75.46	10.17	86.38	96.13	80.41
	Thyme oil	20 ml/l	22.76	72.69	11.50	84.59	113.8	76.81
	Opus	0.75 cm^3/l	09.83	88.21	3.41	95.44	51.29	89.55
Control	Untreated	Only water	83.35	0.00	74.65	0.00	490.8	0.00
LSD at 5%			1.12		0.88		2.65	
Giza 2000	Blight stop	1 l/100 l	10.12	87.83	3.52	95.20	52.20	89.14
	Black seeds oil	20 ml/l	14.5	82.56	4.15	94.34	85.40	82.24
	Eucalyptus oil	20 ml/l	20.22	75.68	10.16	86.15	95.70	80.09
	Thyme oil	20 ml/l	21.85	73.72	11.45	84.39	112.80	76.54
	Opus	0.75 cm^3/l	9.33	88.78	3.40	95.36	51.10	89.37
Control	Untreated	Only water	83.15	0.00	73.35	0.00	480.75	0.00
LSD at 5%			0.98		0.86		2.64	

Table 5b: Effect of spraying different eco-friendly treatments and fungicide on disease severity percentage, average coefficient of infection (ACI) and area under disease progress curve (AUDPC) of *Blumeria graminis* f.sp. *hordei* on barley varieties Giza 123 and Giza 2000 under field conditions during the 2021/2022 growing season.

Barley varieties	Treatments	Concentrations	Disease severity (%)	Reduction (%)	ACI	Reduction (%)	AUDPC	Reduction (%)
Giza 123	Blight stop	1 l/100 l	9.44	88.63	3.50	95.22	52.10	89.05
	Black seeds oil	20 ml/l	14.45	82.60	4.14	94.35	85.35	82.06
	Eucalyptus oil	20 ml/l	20.12	75.77	10.00	86.35	95.55	79.91
	Thyme oil	20 ml/l	21.33	74.32	11.33	84.53	112.78	76.29
	Opus	0.75 cm^3/l	9.22	88.90	3.20	95.63	51.00	89.28
Control	Untreated	Only water	83.05	0.00	73.25	0.00	475.63	0.00
LSD at 5%			1.10		0.76		2.62	
Giza 2000	Blight stop	1 l/100 l	9.35	88.71	3.45	95.28	51.89	89.09
	Black seeds oil	20 ml/l	14.23	82.82	4.22	94.22	85.16	82.10
	Eucalyptus oil	20 ml/l	20.1	75.74	9.70	86.72	95.33	79.96
	Thyme oil	20 ml/l	21.22	74.39	11.18	84.70	112.47	76.35
	Opus	0.75 cm^3/l	9.10	89.02	3.12	95.73	51.28	89.22
Control	Untreated	Only water	82.85	0.00	73.05	0.00	475.63	0.00
LSD at 5%			0.96		0.74		2.61	

On the barley cultivars Giza 123 and Brief 2000, during the two subsequent seasons of 2020/21 and 2021, the fungicide Opus superior to control treatments in regard to effectiveness in reducing the severity of powdery mildew disease, the average infection coefficient (ACI), and the area under the disease progression curve (AUDPC). Blight stop was

the next most effective treatment. On the other hand, the therapy that involved spraying barley plants with an extract of thyme oil was the least effective. The results show that throughout the two research seasons, cultivar Giza 123 of barley was more susceptible to powdery mildew disease than cultivar Giza 2000.

3.2.2 The yield components

Data in Tables (6a and 6b) illustrated that, all environmentally friendly treatments, namely Blight-stop as a biocide, three natural oil extracts: black seeds, thyme, and eucalyptus oils, and the chemical pesticide Opus, when sprayed at the recommended concentrations in Table (2) on barley plants of Giza 123 and Giza 2000, resulted in a decrease in the severity of infection and the development of powdery mildew disease, as well as an increase in the weight of a thousand grains (g) and an increase in grain yield/plot (kg) in comparison to the control treatment during the two growing seasons of 2020/2021 and 2021/2022. In addition to spraying the plants with the fungicide Opus, the highest reading in the weight of 1000 grains (g) and the increase in grain yield/plot (kg) for the two barley cultivars were recorded, followed by an increase in the productivity of the biocide Blight-stop compared to the control treatment during the two study seasons.

Table 6a: Effect of spraying different eco-friendly treatments and fungicide on 1000 kernel weight (g) of barley varieties Giza 123 and Giza 2000 under field conditions during growing two seasons 2021/2022 and 2021/2022.

Barley varieties	Treatments	Concentrations	Growing season 2020/2021		Growing season 2021/2022	
			1000 grain weight (g)	Increasing (%)	1000 grain weight (g)	Increasing (%)
Giza 123	Blight stop	1 l/100 l	55.15	23.38	55.30	23.30
	Black seeds oil	20 ml/l	54.31	21.50	54.42	21.34
	Eucalyptus oil	20 ml/l	53.14	18.88	53.51	19.31
	Thyme oil	20 ml/l	51.66	15.57	51.85	15.61
	Opus	0.75 cm^3/l	56.05	25.39	56.10	25.08
Control	Untreated	Only water	44.70	0.00	44.85	0.00
LSD at 5%			0.42		0.52	
Giza 2000	Blight stop	1 l/100 l	55.25	22.51	55.60	22.98
	Black seeds oil	20 ml/l	54.52	20.89	54.77	21.15
	Eucalyptus oil	20 ml/l	53.52	18.67	53.92	19.27
	Thyme oil	20 ml/l	52.03	15.37	52.38	15.86
	Opus	0.75 cm^3/l	57.10	26.61	57.20	26.52
Control	Untreated	Only water	45.10	0.00	45.21	0.00
LSD at 5%			0.40		0.51	

Table 6b: Effect of spraying different eco-friendly treatments and fungicide on green yield/plot (kg) of barley varieties Giza 123 and Giza 2000 under field conditions during growing two seasons 2021/2022 and 2021/2022.

Barley varieties	Treatments	Concentrations	Growing season 2020/2021		Growing season 2021/2022	
			Grain yield/plot (kg)	Increasing (%)	Grain yield/plot (kg)	Increasing (%)
Giza 123	Blight stop	1 l/100 l	5.15	25.00	5.18	23.63
	Black seeds oil	20 ml/l	4.92	19.42	4.96	18.38
	Eucalyptus oil	20 ml/l	4.81	16.75	4.83	15.27
	Thyme oil	20 ml/l	4.64	12.62	4.70	12.17
	Opus	0.75 cm^3/l	5.23	26.94	5.25	25.30
Control	Untreated	Only water	4.12	0.00	4.19	0.00
LSD at 5%			1.10		0.76	
Giza 2000	Blight stop	1 l/100 l	5.16	22.86	5.18	22.75
	Black seeds oil	20 ml/l	4.98	18.57	5.02	18.96
	Eucalyptus oil	20 ml/l	4.84	15.24	4.87	15.40
	Thyme oil	20 ml/l	4.77	13.57	4.80	13.74
	Opus	0.75 cm^3/l	5.24	24.76	5.26	24.64
Control	Untreated	Only water	4.20	0.00	4.22	0.00
LSD at 5%			0.96		0.74	

On the contrary, the thyme oil extract treatment was the least effective. Provided data in Table (7), indicate that all ecofriendly treatments *i.e.* Blight-stop as a biocide, three natural oil extracts - black seeds, thyme, and eucalyptus oils, and the fungal pesticide Opus, when sprayed at the recommended concentrations in Table (2) on barley plants of Giza 123 and Giza 2000, increased total chlorophyll and carotenoids (mg/g) as estimated the photosynthetic pigments compared with control plants during the growing seasons 2020/21 and 2021/22. The fungicide Opus provided the highest level of total chlorophyll and carotenoids (mg/g) in the majority of cases, followed by biocide-Blight stop. In contrast, thyme oil was the smallest effectiveness. The results showed that throughout the two research seasons, cultivar Giza 123 of barley was more susceptible to the powdery mildew disease and recorded in total chlorophyll and carotenoids (mg/g) less than cultivar Giza 2000.

Table 7: Effect of spraying different eco-friendly treatments and fungicide on total chlorophyll and carotenoids (mg/g) of barley varieties Giza 123 and Giza 2000 under field conditions during growing two seasons 2021/2022 and 2021/2022.

Barley varieties	Treatments	Concentrations	Growing season 2020/2021			Growing season 2021/2022		
			Total chlorophyll (mg/g)		Carotenoids (mg/g)	Total chlorophyll (mg/g)		Carotenoids (mg/g)
			a	b		a	b	
Giza 123	Blight stop	1 l/100 l	3.1	3.0	2.9	3.2	3.1	3.0
	Black seeds oil	20 ml/l	2.6	2.5	2.4	2.7	2.6	2.5
	Eucalyptus oil	20 ml/l	2.3	2.2	2.1	2.4	2.3	2.1
	Thyme oil	20 ml/l	1.7	1.6	1.5	1.7	1.6	2.0
	Opus	0.75 cm^3/l	3.2	3.1	3.0	3.3	3.2	3.1
Control	Untreated	Only water	1.3	1.2	0.9	1.3	1.0	1.1
LSD at 5%			0.42	0.24	0.23	0.22	0.27	0.25
Giza 2000	Blight stop	1 l/100 l	3.3	3.2	3.0	3.4	3.3	3.1
	Black seeds oil	20 ml/l	2.7	2.6	2.5	2.8	2.7	2.5
	Eucalyptus oil	20 ml/l	2.4	2.3	2.2	2.5	2.4	2.3
	Thyme oil	20 ml/l	1.8	1.7	1.6	1.9	1.8	1.7
	Opus	0.75 cm^3/l	3.4	3.3	3.1	3.6	3.5	3.3
Control	Untreated	Only water	1.3	1.2	1.0	1.4	1.3	1.2
LSD at 5%			0.40	0.32	0.30	0.28	0.33	0.31

4. Discussion

The main idea of this research is to utilize the techniques that are the most efficient and environmentally friendly during a certain stage of the farmed plant's development. The environmentally friendly materials as alternative of non-chemical control methods, such as plant extract oils and biological control, to be able to decrease the use of chemical fungicides for controlling barley powdery mildew to produce barley grains with high quality and quantity without any toxicity at the food chain, increasing the fertility of soil, keeping the environment cleaning and maintain the sustainability development over the long term (Matyjaszczyk, 2015; Newton et al., 2010). Obtained data and came to the conclusion the use of biocide, plant oils as Chemical inducers could be utilized as an efficient and safe way to manage powdery mildew disease of barley plants. The principle of sustainable agriculture, which bases plant protection on economic viability, environmental friendliness, and social acceptance, is to grow barley in a range of mixtures. In light of this, modern systems of cultivated plant protection should include any viable techniques for fungus management. In this case using twice spraying of eco-friendly treatments such as Blight stop, thyme, and Eucalyptus oils for controlling barley powdery mildew, in addition compared with traditional systemic Opus fungicide on barley seedlings

of the varieties Giza 123 and Giza 2000 under greenhouse condition. Blight Stop (*Trichoderma harzianum*) when used as a foliar spray (10 ml/l) was the greatest effectiveness in disease control of barley powdery mildew, followed by black seeds oil. On the contrary thyme oil with concentration (20 ml/l) was the least impact one compared to other treatments. Trichoderma species have the ability to directly affect target fungi through mycoparasitism, antibiosis, and competition as their primary strategies for suppressing plant pathogenic fungus (Ahmed, 2018). Although, all the tested compounds clearly minimized the mildewed area with varying degree of disease severity percentage, prolonged both incubation and latent periods which are considered the main components of partial resistance (Wilson, 1994). The essential oils are rich bioactive material such as antimicrobial and these compounds are responsible for the aroma and flavor characteristics which consider the most effective inhibitors of the microbial growth and having a good activity when direct contacted with pathogens (Ahmed, 2013). To put it another way, the foliar spray of Opus (Epoxiconazole) which is among the systemic fungicides under greenhouse conditions shows that the disease was completely checked compared to others. Field application to evaluate the effectiveness of Blight-stop as a biocide, three natural oil extracts: black seeds, thyme and eucalyptus oils, as well as Opus as chemical pesticide treatments against the natural infection with powdery mildew on the susceptible Egyptian barley varieties, field experiments were conducted at Giza Experimental Station, Agricultural Research Centre (ARC), Egypt during the 2020/2021 and 2021/2022 growing seasons. The analyzed of obtained data during the two seasons proved in general that the use of different ecofriendly treatment and fungicide resulted in a significant reduction in disease severity percentage, area under disease progress curve (AUDPC) and average coefficient of infection (ACI) compared to the control treatment. The highest reduction was recorded by opus fungicide followed by Blight stop biocide, whereas thyme oil was recorded the lowest disease protection compared with other treatments. Although the ecofriendly treatments proved less efficient at controlling powdery mildew disease than the fungicides under this investigation, they are less persistent, thus being environmentally safer for human than fungicides. Consequently, it's crucial to pay more attention to basil and other oils that are effective against the microorganisms that cause powdery mildew as safe and adequate alternative control measures for the production of healthy and organic foods (Hafez et al., 2014). In the light of the routine and wide application of these fungicides leave chemical residues in soil, water and grains (Singh et al., 1996) and subsequently may affects animal and human health (Kúc, 1995). Due to its large amount of residues fungicide pollution has become a global environmental problem and found to be highly toxic to aquatic organisms (Haque & Oine, 2019). Control of powdery mildew in this work went along with a sufficient increase in grain yield components of the treated barley plants (Giza 123 and Giza 2000) where there was a direct relationship between the disease infection and yield (Narelle & Piotr, 2021). Additionally, the treatments were successful in that they considerably boosted the yield characteristics compared to the control (Hafez et al., 2019). Presented data in this study, indicate that all ecofriendly treatments and the fungal pesticide increased total chlorophyll and carotenoids (mg/g) as estimated the photosynthetic pigments compared with control treatment during the growing seasons 2020/2021 and 2021/2022. The fungicide Opus provided the highest level of total

chlorophyll and carotenoids (mg/g) in the majority of cases, followed by biocide-Blight stop. In contrast, thyme oil was the smallest effectiveness. This increase could be the result of promoting pigment development, which would increase the effectiveness of the photosynthetic machinery and reduce in phosphorylation rate that usually occur after infection with greater probability of disease resistance (Keutgen & Roeb, 1996). Moreover, the application of some natural treatments was found to increase potassium content, This might multiply the amount of chloroplasts in each cell or stimulate the production of carotenoids, which guard chlorophyll against oxidation.

5. Conclusion

The major goal of this investigation to assess the efficiency of eco-friendly treatments *i.e.*, biocide Blight stop, three natural oil extracts: black seeds, thyme and eucalyptus oils, as well as fungicide Opus in decreasing powdery mildew infection on the susceptible Egyptian barley varieties to assess the efficiency of were conducted in the field conditions at Giza Experimental Station, Agricultural Research Centre (ARC), Egypt during the 2020/2021 and 2021/2022 growing seasons. All eco-friendly treatments and fungicide reduced diseases severity %, area under disease progress curve (AUDPC) and average coefficient of infection (ACI) compared to the control treatment in the two seasons, in addition increase the yield, total chlorophyll and carotenoids compared to the control treatment.

References

Abdullaev RA, Lebedeva TV, Alpatieva NV, Batasheva BA, Anisimova IN, Radchenko EE, 2021. Powdery mildew resistance of barley accessions from Dagestan. *Vavilovskii zhurnal genetiki i selektsii* **25**(5): 528–533.

Agostinetto L, Casa RT, Bogo A, Sachs C, Reis EM, Kuhnem PR, 2014. Critical yield-point model to estimate damage caused by brown spot and powdery mildew in barley. Ciência Rural **44**: 957–963.

Ahmed MFA, 2013. Studies on non-chemical methods to control some soil-borne fungal diseases of bean plants *Phaseolus vulgaris* L. Ph.D. Thesis. Faculty of Agriculture, Cairo University, Cairo, Egypt, p. 137.

Ahmed MFA, 2018. Evaluation of some biocontrol agents to control Thompson seedless grapevine powdery mildew disease. Egyptian Journal of Biological Pest Control **28**(93): 694–700.

Ahmed MFA, Ahmed MSM, Abd El-Aziz Mervat G, 2021. Influence of biological control on sweet pepper powdery mildew disease and its impact on growth and yield under greenhouse condition. The Future of Agriculture **3**: 52–63.

Ali BH, Blunden G, 2003. Pharmacological and toxicological properties of Nigella sativa. Phytotherapy Research **17**(4): 299–305.

Andrres MW, 1982. Latent period and slow rusting in the *Hordeum vulgare* L. *Puccinia hordei* Otth host – parasite system. Ph.D. Thesis, University of Minnesota. St. Poul., USA. p. 113.

Bourras S, Praz CR, Spanu PD, Keller B, 2018. Cereal powdery mildew effectors: a complex toolbox for an obligate pathogen. Current Opinion in Microbiology **46**: 26–33.

Duncan J, 1995. Neural mechanisms of

selective visual attention. Annual Review of Neuroscience **18**: 193–222.

Haque AUL, Oino I, 2019. Managerial challenges for software house related to work, worker and workplace: Stress reduction and sustenance of human capital. Polish Journal of Management Studies **19**(1): 170–189.

Hafez YM, El-Baghdady NA, 2013. Role of reactive oxygen species in suppression of barley powdery mildew fungus, *Blumeria graminis* f.sp. *hordei* with benzothiadiazole and riboflavin. Egyptian Journal of Biological Pest Control **23**(1): 125–132.

Hafez YM, Mourad RY, Mansour M, Abdelaal KhAA, 2014. Impact of non-traditional compounds and fungicides on physiological and biochemical characters of barely infected with *Blumeria graminis* f. sp. *hordei* under field conditions. Egyptian Journal of Biological Pest Control **24**(2): 445–453.

Hafez Y, Abdelfatah A, El-Nashar F, Badr M, Elkady S, 2019. Management of barley net blotch using *Trichoderma asperellum* (T34), eugenol, non-traditional compounds and fungicides. Egyptian Journal of Biological Pest Control **29**: 88.

Holliday P, 2001. A dictionary of plant pathology. Cambridge University Press, Cambridge, UK, p. 536.

Jensen HP, Christensen E, Jørgensen JH, 1992, Powdery mildew resistance genes in 127 Northwest European spring barley varieties. Plant Breeding **108**(3): 210–228.

Keutgen N, Roeb GW, 1996. Effects of stem rust infection (*Puccinia graminis* f. sp. *tritici*) on assimilate partitioning in wheat plants and responses to a fungicide treatment. Journal of Plant Diseases and Protection **103**: 346–352.

Kúc J, 1995. Phytoalexins stress metabolism and disease resistance in plants. Annual Review of Phytopathology 33: 275–297.

Large EC, 2007. Growth stages in cereals illustration of the Feekes scale. Plant Pathology **3**(4): 128–129.

Lichtenthaler HK, 1987. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Methods in Enzymology **148**: 350–382.

Malcolmson L, Nowkirk R, Carson G, 2005. Expanding opportunities for barley food and feed through product innovation. Feed and Food Quality; 18th National American Barley Research Workshop 4th Canadian Barley Symposium, pp. 2–4.

Matyjaszczyk E, 2015. Prevention methods for pest control and their use in Poland. Pest Management Science **71**: 485–491.

Nair KRS, Ellingboe AH, 1965. Germination of conidia of *Erysiphe germinis* f.sp. *tritici*. Phytopathology **55**: 365–368.

Nancarrow N, Aftab M, Hollaway G, Rodoni B, Trębicki P, 2021. Yield losses caused by barley yellow dwarf virus-PAV infection in wheat and barley: A three-year field study in South-Eastern Australia. Microorganisms **9**(3), 645.

Newton JM, Jolly BC, Ockerby CM, Cross WM, 2010, Clinical learning environment inventory: Factor analysis. Journal of Advanced Nursing **66**(6), 1371–1381.

Pandey HN, Amenon TCM, Rao MV, 1989. A Simple formula for calculating area under disease progress curve. Rachis (ICARDA) **8**(2): 38–39.

Pathan AK, Park RF, 2006. Evaluation of seedling and adult plant resistance to leaf rust in European wheat cultivars. Euphytica **149**: 327–342.

Singh T, Harjit Singh, Dhaliwal HS, Datta R, Pannu PPS, 1996. Identification and transfer of powdery mildew resistance from T. dicoccum and related species. In: Abstracts of paper of National Symposium on "Management of Threatening Plant Diseases of National Importance", Indian Phytopathological Society, Ludhiana, India, p. 51.

Stubbs RW, Prescott JM, Saari EE, Dubin HJ, 1986. Cereal Disease Methodology Manual. International Maize and Wheat Improvement Center (CIMMYT), Mexico, p. 46.

Towsend GK, Heuberger TW, 1943. Methods for estimating losses caused by diseases in fungicidal experiments. Plant Disease Reporter **27**(17): 340–343

Tratwal A, Bocianowski J, 2014. *Blumeria graminis* f.sp. *hordei* virulence frequency and the powdery mildew incidence on spring barley in the Wielkopolska province. Journal of Plant Protection Research **54**(1): 28–35.

Wang Y, Zhang G, Wang F, Lang X, Zhao X, Zhu J, Hu C, Hu J, Zhang Y, Yao X, Liu H, Ma T, Niu Y, Wang Z, Feng J, Wangmu, Lin R, 2023. Virulence variability and genetic diversity in *Blumeria graminis* f. sp. *hordei* in Southeastern and Southwestern China. Plant Disease, **107**(3): 809–819.

Wilson JP, 1994. Field and greenhouse evaluation of pearlimillet for partial resistance to *Puccinia substraiata* var. *indica*. Plant Disease **78**: 1202–1205.

Xu Z, Duan X, Zhou Y, 2014. Population genetic analysis of *Blumeria graminis* f.sp. *tritici* in Qinghai Province, China. Journal of Integrative Agriculture **13**: 1952–1961.

Yu K, Lenz-Wiedemann V, Chen X, Bareth G, 2014. Estimating leaf chlorophyll of barley at different growth stages using spectral indices to reduce soil background and canopy structure effects. ISPRS Journal of Photogrammetry and Remote Sensing **97**: 58–77.