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NANO-SILVER AND NON-TRADITIONAL COMPOUNDS MITIGATE THE ADVERSE EFFECTS OF NET BLOTCH DISEASE OF BARLEY IN CORRELATION WITH UP-REGULATION OF ANTIOXIDANT ENZYMES

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Abstract

Exogenous application of nano-silver, non-traditional compounds and fungicides were used to alleviate the harmful effect of net blotch disease in the highly susceptible Egyptian barley 'Giza 2000'caused by *Pyrenophora teres* L. The symptoms of net blotch disease were significantly dwindled as a result of foliar spray with fungicides such as Montero, Belize and Cabri Top. Application of Tilt, Vitavax, Nano-silver, Allicin and Benzothiadiazole (BTH) fungicides moderately controlled the effects of disease severity. While, fungicides Premis, Eugenol and Oxalic acid treatments did not reduce significantly the severity of net blotch disease. As a result of these treatments, the activities of antioxidant enzymes activity were increased significantly as compared with the untreated control plants. The tested treatments were effective, since the electrolyte leakage percentage of treated plants decreased significantly, while the yield attributes were increased significantly as compared with control. The maximum1000-grain weight (g), grain yield (kg ha^{-1}) and biological yield (kg ha^{-1}) were achieved with the application of fungicide 'Montero' followed by 'Belize'. Therefore, the novel findings of the present study may be supportive to farmers and plant breeders with non-traditional compounds and basic mechanisms to create new resistant barley cultivars, consequently, decreasing fungicides use and environmental pollution.

Key words: Barley; Nano-silver; Antioxidant enzymes; Fungicides; Yield.

Introduction

Barley (*Hordeum vulgare* L.) is an imperative cereal crop in the world and ranked fourth after wheat, maize and rice. It is cultivated in several areas including Tibet, Nepal, Ethiopia, and the Andes on mountain slopes. In North Africa, Pakistan, the Middle East, Eritrea, Afghanistan and Yemen, barley is cultivated as a rain-fed crop (Anon., 2014). In developed countries, the crop is also considered the most important species for animal feed, malting and exportation. Furthermore, previous reports indicate that barley has been used for various scientific studies including biotechnology, plant breeding methodology, pathology, genetics, virology and cytogenetics (Hockett & Nilan, 1985).

Barley has the potential to become one of the essential cereal crops in Egypt.Various biotic and abiotic stresses significantly influence the growth and development of plants, leading to a reduction in crop yields (Jahan *et al.,* 2019; EL Sabagh *et al.,* 2019). However, net blotch disease caused by *Pyrenophora teres* leads to serious yield losses and quality reduction in Northern Egypt. Recently, the disease has become an ever-endemic disease to most parts of the north in the country (salt affected fields and rain areas) where barley is important. The disease is reported to flourish well under high relative humidity and warm environments with temperatures ranging from 15 to 25°C (Krupinsky *et al*., 2002). New epidemics of the net blotch have been noted lately around the world as reported by Liu & Friesen (2010). Furthermore, the yield losses of about 10% to 40% and up to 100% in harsh scenarios have been reported. Control measures such as use of crop rotation, stubble destruction, nutrition, application of fungicides and use of resistant varieties and healthy grains have been adopted (Thomason *et al*., 2005). Few research articles on net blotch disease in Egypt have been published (Hafez *et al*., 2016). Despite the wide distribution of the disease in the country, susceptibility of several landless and improved varieties, and substantial grain yield losses, research on net blotch epidemiology and management is limited. Moreover, the disease still poses a serious challenge especially in high and medium altitude areas in Egypt. In this current context, the aim of this study was to achieve an improvement of the growth and yield components of barley plants infected with net blotch disease as well as find out new and alternative control strategies using nano-silver, non-traditional compounds and fungicides in highly susceptible Egyptian barley variety 'Giza 2000'.

Materials and Methods

Plant materials and field experiments: The efficiency of six fungicides (Premis, Tilt, Montero, Belize, Cabri Top, Vitavax) and non-traditional treatments [Nano-silver, Allicin, Eugenol, Tannic acid, Oxalic acid and benzothiadiazole (BTH)] were assessed against the natural infection with net

blotch on the susceptible Egyptian barley variety Giza 2000. Two field experiments were conducted at the Experimental Farm of Sakha Agricultural Research Station, Egypt, during the two successive seasons 2015-16 and 2016-17. The experimental soil was categorised as clay loamy, with an average pH of 8.2 and electrical conductivity (EC) of 210 dS m^{-1}. Grains were hand drilled at the recommended seeding rate of barley in Egypt (119 kg ha^{-1}). Each plot (4.2 m^2) was sown in six rows of 3.5 m long, with 20 cm between rows. Randomized complete block design with three replicates were laid out for all the experiments. Traditional and cultural practices were applied at proper time per recommendations by Ministry of Agriculture (Egypt). Premis and Tilt were used as grains treatments, while the other treatments as foliar spray. Barley grains were sown on 15th December and the preceding crop was maize in both seasons. All foliar spray treatments were applied twice at the beginning of infection and the second was after 10 days. Disease severity was recorded first time at the beginning of March and repeated six times at 5-day intervals. Plant height, spike length, grain weight per spike (g) and number of grains per spike were estimated as the average weight of 10 random spikes from the central rows. Biological yield (kg ha^{-1}.) was recorded for all harvested plants/plot and converted to kg ha^{-1}. Grain yield (kg ha^{-1}) was noted and measured from the grains of harvested plants/plot after threshing and then converted to kg/ha. The 1000-grain weight (g) was determined by the mean weight of random samples of 1000 grains.

Pathogenic fungal inoculation: Natural infection with *Pyrenophora teres f. teres* conidia, the causal of barley net blotch, was conducted under field condition. Plant spreaders (susceptible host 'Giza 2000') were uniformly inoculated with freshly collected conidiospores by placing heavy infected plants of barley sensitive to *P. teres* inoculation.

Experimental design and treatments: The experiments were conducted in randomized complete plot design (RCPD) with three replicates for each treatment. Fungicides as well as non-traditional compounds were used in the present study. Specifically, the fungicides Premis, Tilt 25%, Cabrio top 60% WG, Belize 38% WG, Montero 30%, and Vitavax were studied. The fungicides were produced by Shoura Chemical Company, Kafr El-Zayat chemical Company Limited, Delta Agency and Trade, Cairo, Egypt. Silver nanoparticles (NPs) (99.99%) were obtained from Nanotech Company Limited, Cairo, Egypt. An aqueous solution (0.9 mM, 50% w/w) benzo-(1, 2, 3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH), (Syngenta, USA) was ectopically applied by spraying all the plants using a pump aerosol sprayer Infected plants (without treatments) served as controls. Similarly, 0.5g/L of Allicin, 1g/ L oxalic acid and tannic acid were sprayed on all the plants except the infected ones. For the control, leaves were inoculated only with the pathogen. The grains were treated directly with Premis, Tilt, Capri Top and Montero fungicides before sowing. For Belize and other treatments, the leaves were sprayed twice during the first appearance of infection and 10 days later with belize fungicide, Vitavax, Nano Silver, Allicin, Eugenol, Tannic acid (TA), Oxalic acid (OXA) and benzothiadiazole (BTH).

Disease severity assessments: A scale of 0-9 was used for disease severity assessment, with 0 representing no infection and 9 when all barley leaves dried due to infection by the fungus. The adult plants response data was scored at 65, 70, 75, 80, 85 and 90 days for assessment of disease severity. Consequently, the following ranges and code for assessment of infection were purposively used: 0-3 for disease resistant, 4-5 for moderately resistant, 6-7 for moderately susceptible, and 8-9 for susceptible.

Electrolyte leakage: Measurements of electrolyte leakage were performed after 15 days in line with previous researchers with some modifications (Szalai *et al*., 1996; Whitlow *et al*., 1992). In summary, 20 leaf discs (1 cm) of barley leaves were placed into individual flasks, containing 25 ml deionized water produced using standard equipment (Milli-Q 50, Millipore, USA). With the aid of an automated shaker, the flasks were shaken for 20 hrs at ambient temperatures. This conducted to aid electrolyte leakage from injured tissues of the plants. Measurements for initial electrical conductivity ($EC_{initial}$) were noted and recorded for each vial using an Acromet AR20 EC meter (Fisher Scientific, Chicago, USA). The flasks were then immersed in a hot water bath (Fisher Isotemp, Indiana, PA) at 80°C for 1hr to induce cell rupture. The vials were once more placed on the shaker (Innova 2100 platform) for 20 hrs at 21°C before measuring the final conductivity (EC_{final}) for each flask. Electrolyte leakage percentage (EL%) for each bud was calculated by dividing EC_{final} with $EC_{initial}$, the result multiplied by 100. Electrolyte leakage was measured 15 days after the appearance of the infection.

Detection of reactive oxygen species levels: The reactive oxygen species (ROS) such as assuperoxide ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) levels were determined by the purple coloration of nitro blue tetrazolium (NBT) and a reddish-brown coloration of 3,3-diaminobenzidine (DAB) respectively. The ROS were measured within the 7 days of treatment (1, 2, 4 and 7 days). While barley leaves were vacuum infiltrated with potassium phosphate buffer solution (10 mM, pH 7.8) containing 0.1 w/v % NBT (Sigma-Aldrich, Steinheim, Germany) in line with previous methods (Ádám *et al*., 1989). The NBT-treated samples were incubated under daylight for 20 min and then cleared in 0.15 w/v % trichloroacetic acid in ethanol: chloroform 4:1 v/v for 1 day (Hückelhoven *et al*., 1999). Then, samples were carefully washed with distilled water and placed in 50% glycerol prior for assessment. The discoloration of the leaf discs resulted by NBT staining was quantified with the aid of a ChemiImager 4000 digital imaging system (Alpha Innotech Corp, USA). The treatment and test procedures were repeated three times.

Biochemical assays of antioxidant enzymes: To evaluate enzyme assays in plants, 0.5 g leaf material was homogenized at 0 to 4°C in 3 ml TRIS buffer (50 mM, pH 7.8), made from 1 mM EDTA-Na_2 and 7.5% polyvinylpyrrolidone. The homogenates were centrifuged at 12,000 rpm and 4°C for 20 minutes and the total

soluble enzyme activities were measured at 25°C using spectrophotometer (Model UV-160A, Shimadzu, Japan). The enzyme assays were measured after 7, 15 and 30 days. Activity of catalase (CAT) was measured using spectrophotometer per Aebi (1984) method. Polyphenol oxidase (PPO) activity was measured in line with the method described by Malik & Singh (1980). Changes in absorbance were recorded at 30-second intervals for a period of 3 min at 495 nm wave length. Peroxidase (POX) activity was directly determined from the crude enzyme extract according to a typical procedure proposed by Hammerschmidt *et al*., (1982). Changes in absorbance for POX were recorded at 30-second intervals for 3 min at 470 nm wavelength. All the enzyme activities were noted and expressed as increase in absorbance per minute per gram fresh weight.

Statistical analysis: Statistical Package for the Social Sciences (SPSS) (2016) and Microsoft Excel (2016) were used for data analyses. The reported data represent the mean ± SD. Student's t-test were used to determine whether significant differences (p<0.05) existed among mean values in line with O'Mahony (1986).

Results and Discussion

Effect of treatments on the disease severity (%): Barley net blotch disease has been widely spread and became a serious problem in untreated fields. Use of fungicides reduces the occurrence of fungal diseases and thereby reduction in yield losses, but increase the economic profit. A typical characteristic of net blotch is a sudden increase in the production of conidia following a prolonged, steady increase in conidia proliferation as the weather remains cool and moist. Infection of barley leaves is the highest when humid conditions persist for 10-30 hrs or longer and the optimal temperature range for infection is 15-25°C (Prigge *et al*., 2004). The presented data in Fig. 1-A, showed that morphological changes in barley leaves after the treatments. Net blotch symptoms were first observed in early March both seasons (2015-16 and 2016-17). The effects of 12 treatments against *P. teres* and untreated control on spring barley variety 'Giza 2000' in both seasons are presented in Table 2 and Fig. 1. Among the fungicides, Montero, Belize and Cabri Top were found the most effective for reducing disease severity. Tilt, Vitavax, Nano-silver, Allicin and benzothiadiazole (BTH) treatments also decreased the disease severity. However, Premis, Eugenol and Oxalic acid showed a slight reduction in net severity (Fig. 1). It is evident that the best disease control effect was achieved with Montero fungicide treatment which significantly reduced the disease severity (%) and disease symptoms in both seasons compared with grains treatment by Premis or non-fungicide treatments (Table 1 and Fig. 1B-C). This effect on disease control may be due to the role of these fungicides in inhibitors of mitochondrial respiration or sterol biosynthesis inhibitors of the fungal cells. These results are similar to the findings of Stepanović *et al*., (2015). BTH treatment gave a medium effect on disease severity. Tannic acid treatment trend showed slightly lower values compared to control in both seasons (Fig. 1B-C).

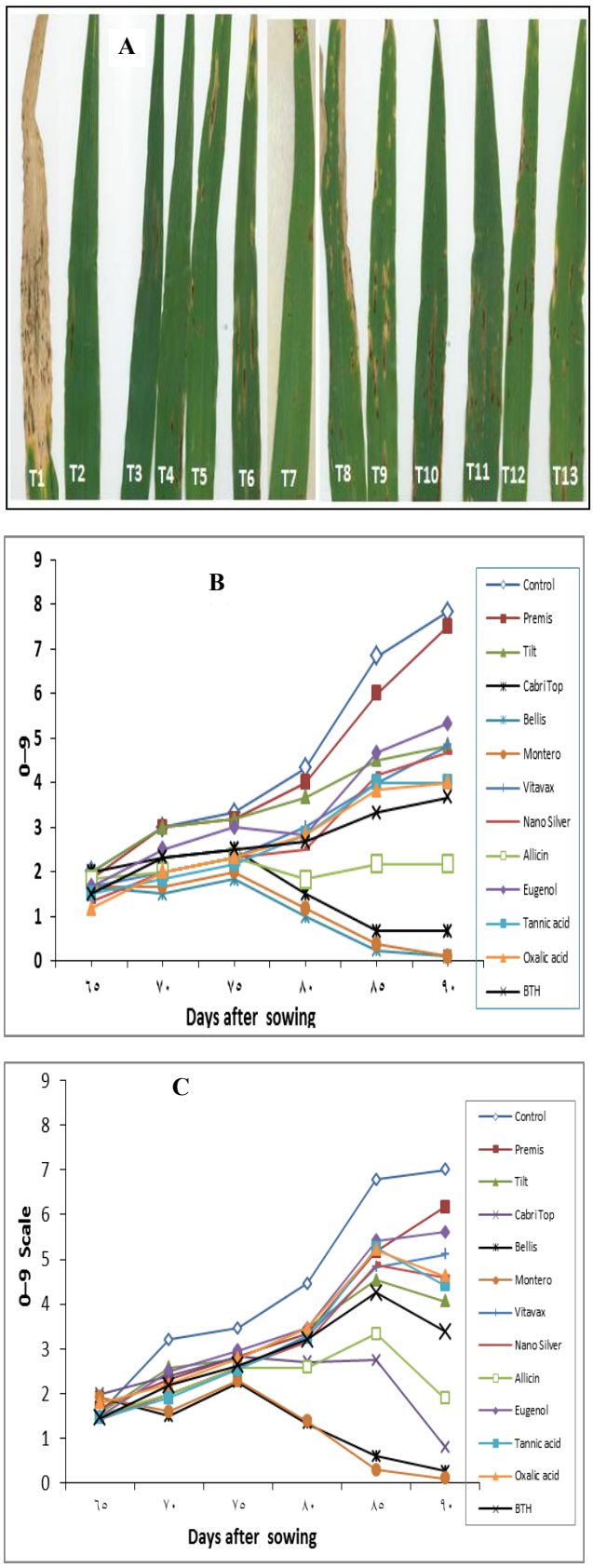


Fig. 1. Effect of treatments on disease symptoms of barley three days after the appearance of natural infection with *Pyrenophora teres* f.sp. *teres*. (A) Morphological symptoms, disease symptoms in2015/2016 (B) and 2016/2017 (C). Control (T1) Premis (T2), Tilt (T3), Cabri Top (T4), Belize (T5), Montero (T6), Vitavax (T7), Nano Silver (T8), Allicin (T9), Eugenol (T10), Tannic Acid (T11), Oxalic Acid (T12), BTH (T13).

Table 1. Impact of treatments on disease symptoms scored at 65, 70, 75, 80, 85 and 90 days after sowing during the two seasons.

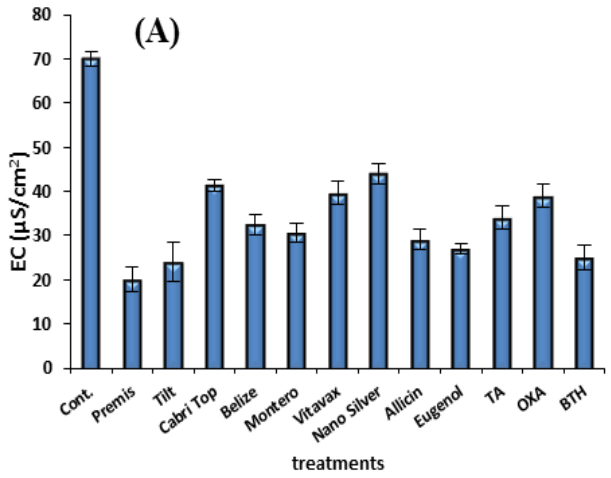
Treatments	2015-16						2016-17					
	65	70	75	80	85	90	65	70	75	80	85	90
Control	2.00	3.00	3.33	4.33	6.83	7.83	1.58	3.21	3.46	4.46	6.79	7.00
Premis	1.83	3.00	3.17	4.00	6.00	7.50	1.67	2.33	2.83	3.33	5.17	6.17
Tilt	2.00	3.00	3.17	3.67	4.50	4.83	1.58	2.58	2.79	3.46	4.54	4.07
Cabri Top	2.00	2.33	2.50	1.50	0.67	0.67	2.00	2.40	2.83	2.71	2.75	0.81
Belize	1.67	1.50	1.83	1.00	0.23	0.10	1.92	1.50	2.25	1.33	0.60	0.27
Montero	1.67	1.67	2.00	1.17	0.37	0.10	1.92	1.60	2.29	1.38	0.30	0.10
Vitavax	1.67	2.00	2.33	3.00	4.00	4.83	1.50	2.00	2.58	3.29	4.83	5.11
Nano Silver	1.33	2.00	2.33	2.50	4.17	4.67	1.42	2.00	2.58	3.17	4.88	4.60
Allicin	1.83	2.00	2.33	1.83	2.17	2.17	1.54	2.00	2.58	2.58	3.33	1.89
Eugenol	1.67	2.50	3.00	2.83	4.67	5.33	1.50	2.50	2.96	3.46	5.42	5.61
Tannic acid	1.50	1.83	2.17	2.83	4.00	4.00	1.46	1.90	2.54	3.25	5.25	4.42
Oxalic acid	1.17	2.00	2.33	2.83	3.83	4.00	1.79	2.21	2.79	3.46	5.21	4.63
BTH	1.50	2.33	2.50	2.67	3.33	3.67	1.46	2.19	2.63	3.21	4.25	3.39
$LSD_{0.05}$	- -	- -	0.83	0.94	1.35	1.62	- -	0.65	0.76	0.84	1.95	1.07
F-test	ns	ns	**	**	**	**	ns	**	*	**	**	**

LSD: Least significant difference. **: Highly significant. *: Significant. ns: Non-significant

Electrolyte leakage: Electrolyte leakage (EL) is an indicator of cell membrane permeability (Szalai *et al*., 1996). Fig. 2A-B show the effect of treatments on electrolyte leakage (cell wall permeability) percentage in barley leaves at 15 days after the appearance of natural infection with *Pyrenophora teres* f. *teres* in two seasons. Infected barley plants with *P. teres* treated with fungicides as well as the non-traditional treatments showed substantial decrease in electrolyte leakage as compared with control, which showed a significant increase of the membrane permeability (Fig. 2A-B). Chemical compounds and biotic or abiotic stresses are reported to alter resistance or susceptibility of plants to infection as a result of their effects on membrane permeability. Furthermore, elevated temperature stress could induce vulnerability in maize through its effect on membrane permeability as noted by high electrolyte leakage (Garraway *et al*., 1989). The results of this study indicated that the treatments protected cell membranes of barley plants during the pathogen attack, while the cell membrane of the control treatment was affected by pathogen infection and lost its constituents. The present results agree with those obtained by previous studies (Abdelaal *et al*., 2018; Hafez *et al*., 2016).

Reactive oxygen species levels and antioxidant enzymes activity: As a result of the different treatments, reactive oxygen species (ROS) mostly superoxide ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) levels were increased early after appearance of infection in stressed barley plants (Fig. 3A-B). The activity of catalase (CAT), peroxidase (POX) and polyphenol oxidase (PPO) enzymes was affected in infected barley plants. Indeed, activities of CAT, POX and PPO were significantly increased in infected barley leaves treated with fungicides and the non-traditional treatments compared with control treatments at 7, 15 and 30 days later after appearance of natural infection under field conditions (Figs. 4 and 5). The early increased levels of ROS after infection could be a cause of suppressing or killing the pathogen particularly at the time point one day after infection (1 dai), consequently, the antioxidant enzymes activity (CAT, POX and PPO) were up-regulated later at 7 dai. Interestingly enough that antioxidant enzyme activities of barley were up-regulated and protected the plants from biotic and abiotic stresses as a result of chemical inducers treatments (Abdelaal, 2015). Similar results were obtained in barley plants infected with net blotch disease suggesting that the increase of ROS levels early after infection could be the cause of killing or inhibiting the pathogen and thereby up-regulation of the antioxidant enzymes (Hafez *et al*., 2016). Furthermore, the infected wheat plants with leaf rust disease and treated with safety resistance inducers showed an increase of ROS and antioxidant enzymes after infection. (Hafez *et al*., 2017). Therefore, the early accumulation of superoxide contributes to symptomless in non-host resistance of plants to bio-trophic pathogens in which thereby, prove the pivotal role of early accumulation of ROS in non-host resistance (Künstler *et al*., 2018).

Results of this work were in accordance with previous studies in which plants were treated with non-traditional compounds under biotic and abiotic stresses (Omara *et al*., 2015; Abdelaal *et al*., 2017; Hafez *et al*., 2017; Helaly *et al*., 2017). The high levels of ROS after infection were stimulated, thus, increasing the antioxidant enzyme activities and immunized the plants against disease infection. Several studies showed salicylic acid (SA) and functional analogues such as BTH and 2,6-dichloroisonicotinic acid could cause ROS accumulation via mitochondrial electron transport inhibition (Norman *et al*., 2004) or antioxidant enzymes (Wendehenne *et al*., 1998; Hafez *et al*., 2008). Other studies also suggested that H_2O_2 induced SA accumulation which then enhanced the buildup of H_2O_2 (Van Camp *et al*., 1998). Because of H_2O_2 and O_2.- buildup, microbursts may produce, intensify and spread the H_2O_2 signal essential for oxidative cell death and formation of systemic acquired resistance. Furthermore, Fodor *et al*., (2001) observed that an early burst of ROS and transient inhibition of antioxidant defense 1-2 days after inoculation of tobacco leaves with TMV, followed by a massive induction of antioxidants. These phenomena explain the observed trends and scenario in our current study.



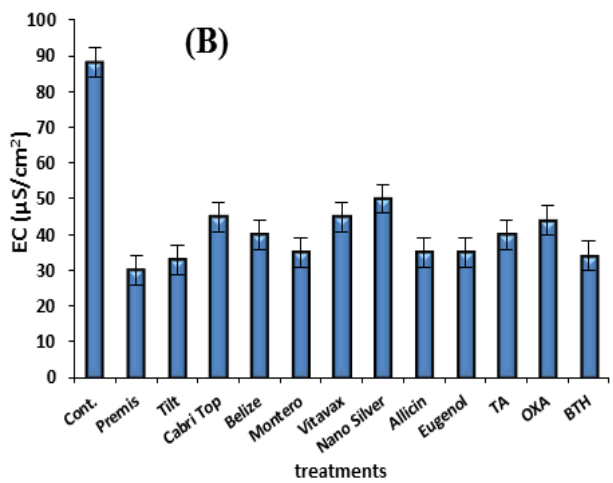
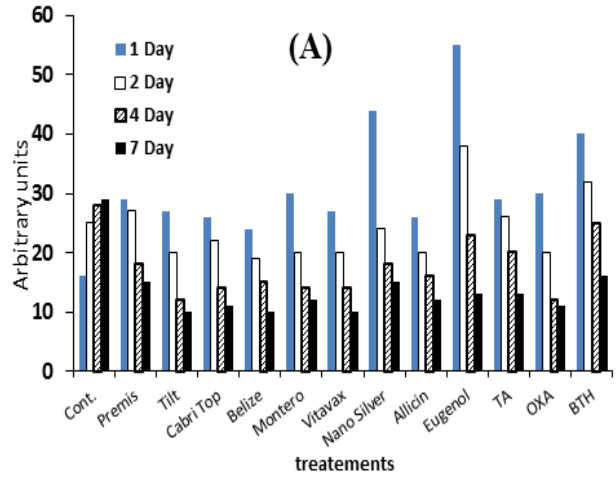


Fig. 2. (A-B): Effects of treatment on electrolyte leakage as an indicator of cell membrane permeability in Barley plants for 2015/2016 (A) and 2016/2017 (B) seasons. Data represent means of 3 measurements in each of two independent experiments ± SD.



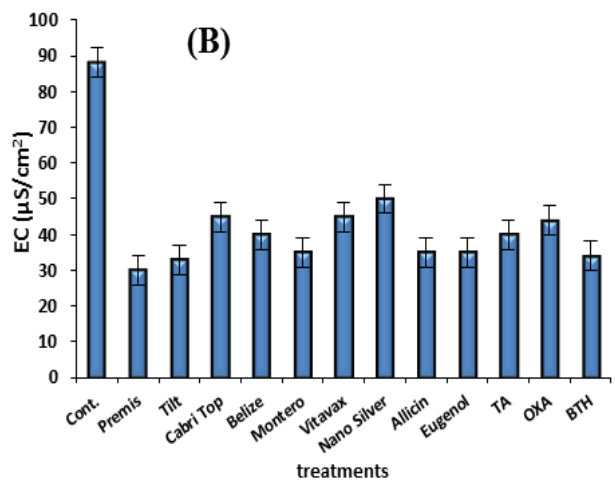


Fig. 3. (A-B): Effect of treatments on superoxide (A) and hydrogen peroxide (B) levels in barley leaves 1, 2, 4 and 7 days after the appearance of natural infection with *Pyrenophora teres* f.sp. *teres*. Data presented as means of 3 measurements in each of two independent experiments ± SD.

Table 2. Plant height, spike length, grain number per spike, grain weight per spike, 1000 grain, biological yield and grain yield weight against treatments in 2015-16 (A) and 2016-17 (B) seasons.

Treatments	Plant height (cm)		Spike length (cm)		grain number per spike		grain weight per spike (g)		1000 grain weight (g)		Biological yield (kg/ha)		Grain yield (kg/ha)	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Control	88.3	84.44	7.0	7.00	50.4	51.13	29.8	28.85	60.2	56.58	8650	3476	3417	1310
Premis	85.0	84.17	7.0	6.58	47.4	47.94	28.7	28.44	62.4	59.34	8310	3366	3200	1222
Tilt	94.3	92.53	6.7	6.81	49.2	51.28	30.1	32.01	60.5	62.39	10367	4377	3450	1414
Cabri Top	94.0	97.50	7.0	6.58	52.8	52.88	34.1	34.04	65.0	64.35	13083	5148	4357	1700
Belize	96.7	92.81	7.3	7.19	54.0	52.44	35.7	34.50	67.7	65.75	13250	5399	4500	1890
Montero	94.3	93.78	7.3	7.19	54.0	52.30	35.7	34.50	67.8	65.75	14793	6424	4667	1953
Vitavax	86.7	89.31	6.7	6.81	46.7	46.89	29.8	28.29	63.6	60.34	10000	4638	3433	1594
Nano Silver	88.3	86.94	7.0	7.00	53.3	51.26	32.6	32.00	60.9	62.46	10450	4074	3833	1377
Allicin	90.0	90.83	7.0	7.00	49.0	47.86	29.6	30.05	62.6	62.90	10900	4421	3683	1471
Eugenol	83.3	90.53	7.3	7.19	51.9	51.50	29.9	29.81	59.8	57.95	9967	4192	3267	1343
Tannic acid	90.0	91.83	7.3	6.78	50.1	49.91	30.9	30.62	60.0	61.67	9500	4270	3167	1476
Oxalic acid	89.0	87.33	7.3	6.78	48.7	48.49	29.8	29.08	60.9	59.98	9750	4226	3367	1262
BTH	88.3	91.11	7.7	7.39	49.4	50.96	30.4	31.57	61.7	61.91	11183	4577	3900	1498
LSD 0.05	6.44	5.97	- -	- -	- -	2.60	2.98	2.15	5.17	4.21	559	516	268	272
F test	**	**	ns	ns	ns	**	**	**	*	**	**	**	**	**

LSD, Least significant difference; **: Highly significant; *, Significant; ns, Non-significant; A = 2015-16 season; B = 2016-17 seasons

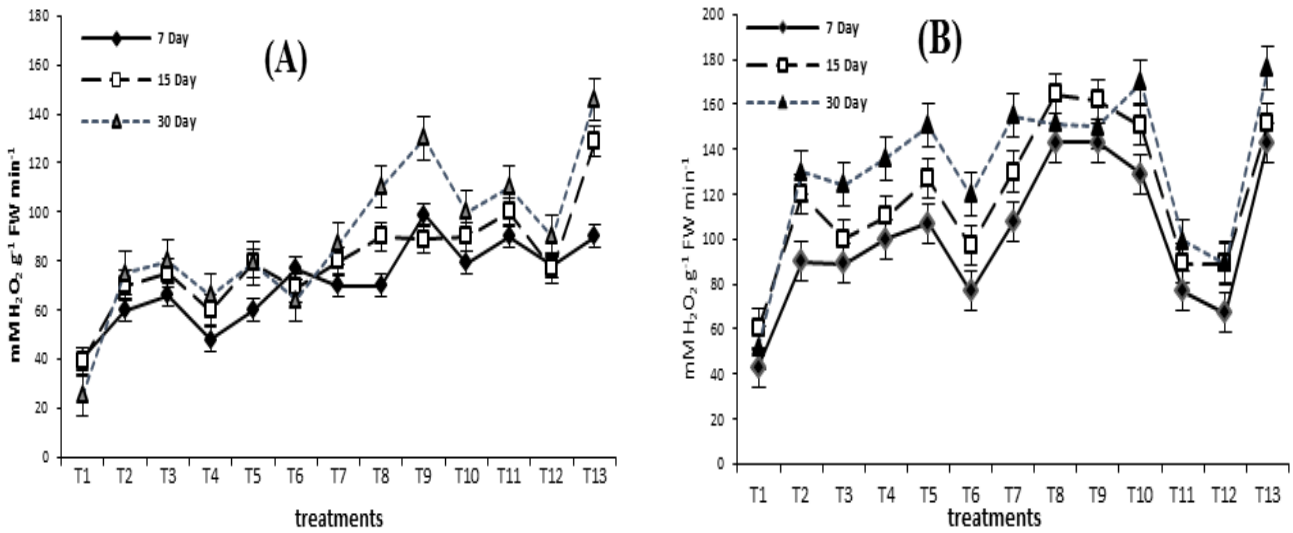


Fig. 4. Effect of treatments on catalase (CAT) enzyme activity of barley plants 7, 15 and 30 days after appearance of natural infection with Pyrenophora teres f. sp. teres during the 2015/2016 (A) and 2016/2017 (B) seasons. Data presented as means of 3 measurements in each of two independent experiments ± SD.

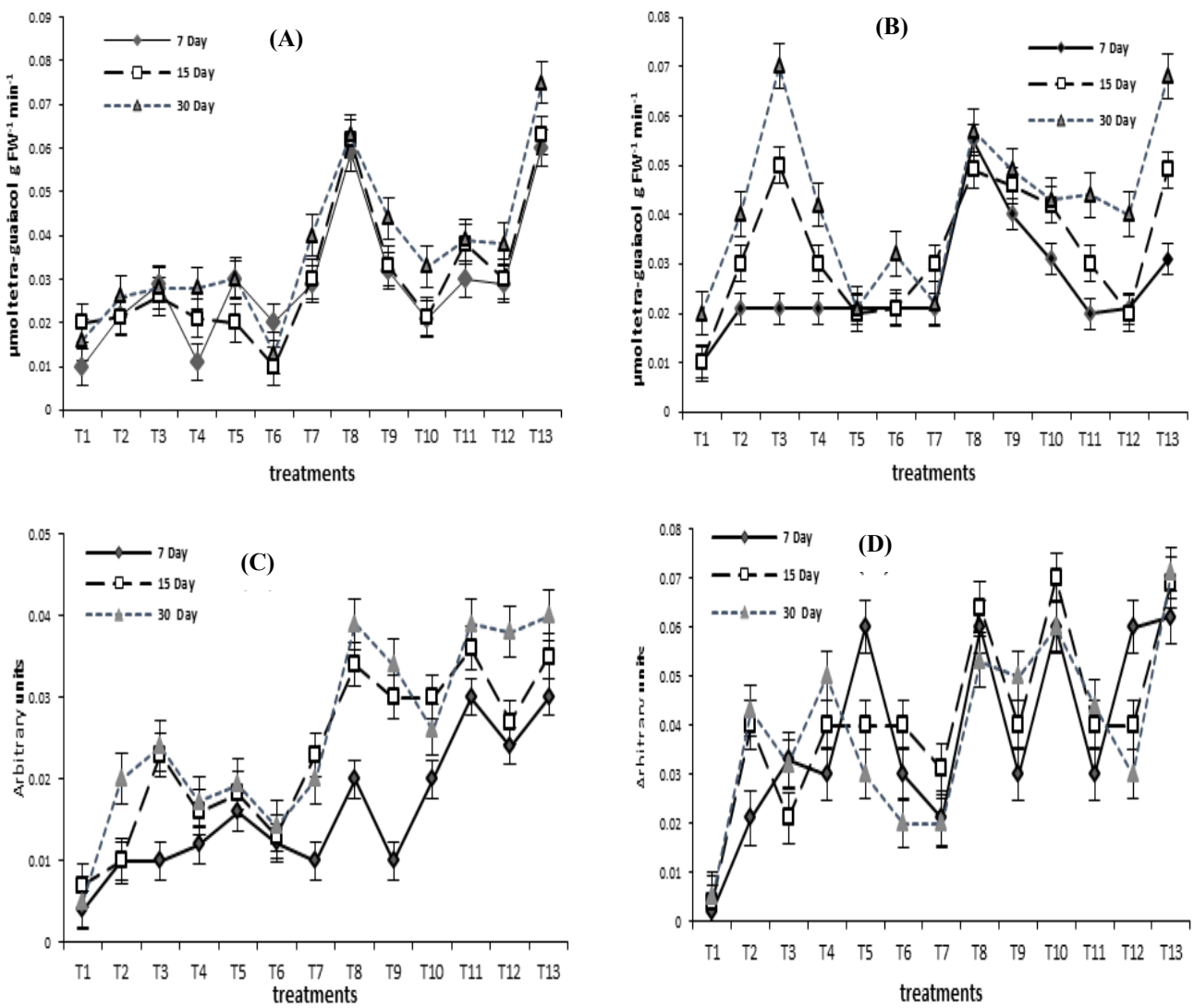


Fig. 5. Effect of treatments on peroxidase (POX) during 2015/2016 (A) and 2016/2017 (B) seasons as well as polyphenol oxidase (PPO) enzyme activity during 2015/2016 (C) and 2016/2017 (D) of barley plants 7, 15 and 30 days after appearance of natural infection with *Pyrenophora teres* f. sp. *teres*. Data presented as means of 3 measurements in each of two independent experiments ± SD.

Effect of treatments on yield characters: It is a well-established fact that plant yield is estimated by plant height, grain number per spike, spike length and 1000 grain weight. These concepts not only involve the final crop yield and components, but also probe into the disease infections that have occurred early in the growth stages causing variation in yield potential.

The results in Table 2 indicated that most of the investigated characters were significantly influenced (p<0.05) by foliar spray treatments in both seasons, especially Montero, Belize and Cabri Top fungicide treatments. The greatest respective 1000-grain weight (g), grain yield (kg/ha) and biological yield (kg/ha) values compared with control were obtained in Montero (67.8 g, 4667 kg/ha, 14793 kg/ha) followed by Belize (67.7 g, 4500 kg/ha, 13250 kg/ha) and Cabri Top treatments (65.0 g, 4357 kg/ha, 13083 kg/ha) in both seasons, respectively (Table 2). The results showed positive effects of fungicide treatments in improving growth and yield of infected barley plants. This valuable effect may be due to that, application of treatments led to improve growth and yield under pathogen stress. These findings are in agreement with previously reported studies by Abdelaal (2015), Abdelaal *et al.*, (2014) and Stepanović *et al*., (2015) in various plants under abiotic and biotic stresses.

Conclusion and recommendations: The study evaluated the efficiency of grains treatment and foliar application of Nano-silver**,** non-traditional compounds and fungicides for controlling the barley net blotch disease in susceptible Egyptian barley 'Giza 2000' caused by *Pyrenophora teres* L. The disease symptoms were significantly decreased as a result of using foliar spray with fungicide treatments of 'Montero', 'Belize' and 'Cabri Top'. Moderate effects on disease severity were observed in plants treated with Tilt, Vitavax, Nano-silver, Allicin and benzothiadiazole (BTH). The activities of antioxidant enzymes were increased significantly as compared with the control. The electrolyte leakage percentage decreased significantly mainly with fungicide treatments compared with control plants. The yield character of the treated barley generally increased as compared with the control. In the current study, most of the treatments could suppress the fungus and improve growth characters of infected barley plants by *P. teres* the causal agent of net blotch disease. According to our knowledge, this work provides novel results that can support plant breeders worldwide to create new resistant barley cultivars and prevent environmental pollution due to use of fungicides which are not only costly but also cause severe human diseases because of their residual harmful effects.

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