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THE POTENTIAL ABILITY OF SOME ABIOTIC AGENTS TO CONTROL BARLEY NET BLOTCH DISEASE

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ABSTRACT

The efficacy of some abiotic agents such as chitosan (CHT), xanthan gum (XAN), propolis (PROP) and propiconazol fungicide at its recommended dose (0.25 ml/l) against barley net blotch disease caused by *Drechslera teres* (*D. teres*) were evaluated. *In vitro* trials, CHT proved high inhibition effect on spore germination than on the linear growth of *D. teres*, while the contrary occurred by PROP. Meanwhile, XAN had not exhibited any direct effect against mycelial growth and spore germinations. *In vivo* trials, under greenhouse conditions, disease responses at both seedling and adult stages were significantly reduced compared to the control. Both tested concentrations of CHT and propiconazol treatments revealed the highest protection level by reducing the number and rate of lesion increase followed by XAN. Treatments of PROP showed the lower protection level especially at seedling stages. The ability of tested substances to trigger physiological defense reaction in plant tissues was investigated during the assessment of some defense related enzyme activities *i.e.* peroxidase (POD) chitinase (CHS) and phenylalanine ammonialyase (PAL). The higher activity of POD was obtained by propiconazol, followed by XAN at 0.3% and PROP at 0.6%. Activities of CHS showed the highest stimulation response with PROP at 0.6%, CHT at 0.1 and 0.15%, followed by XAN at 0.3%. However, the lowest response was recorded with the propiconazol. PAL activity was observed to be high in plants treated with propiconazol followed by CHT at 0.1 and 0.15%.

Key words: Barley, net blotch, *Dereshslear teres*, chitosan, xanthan gum, propolis, PAL, POD.

INTRODUCTION

Barley (*Hordeum vulgare* L.), a member of *Poaceae* (*Graminaceae*) family is one of the major cereal crops and most dominate crops that can be established and growing under hard conditions in which it can be grown successfully and better than any other cereal grains (Newton *et al.*, 2011).

Barley net blotch disease caused by *Drechslera teres* Sacc., (teleomorph *Pyrenophora teres*) is considered a disease of serious concern for barley worldwide especially in cool and moist regions (Hundie *et al.*, 2004; Statkeviciūtė and Leistrumaite, 2010). Net blotch infection losses range between 10 up to 40% (El-Mor *et al.*, 2016). However, under favorable conditions losses can reach 100% (Mathre, 1982).

Since, the repetition of fungicide applications poses a negative environmental and agricultural impacts, therefore the development of alternative strategies for crop protection are required in order to avoid increasing demand of fungicides. Among the most common of these strategies are the uses of natural production derived from organisms (Tripathi and Dubey, 2004). Chitosan is a chitin de-acetylated form, applied to plant as an alternative control approach and their effectiveness has been evaluated against several plant diseases (Atia *et al.*, 2005; El-Hadrami *et al.*, 2010). It has been investigated to induce defense immune system in plant against plant pathogen infections (Atia

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et al., 2005; Faoro *et al.,* 2008; Falcon-Rodriguez *et al.,* 2009; Hadwiger, 2013). Xanthan gum is an intensive polymer produced by *a* fermentation of *Xanthomonas compestris* bacterial. Antifungal activity of xanthan gum compound has been investigated to protect plant from pathogenic fungi (Bach *et al.,* 2003; Castro and Bach, 2004). Propolis is a natural sticky mixture produced by honeybees by substances collected from plants and shown to have such activity against plant pathogenic fungi (Mahdy *et al.,* 2006; Giovanelli, 2008).

Motivation of plant defense resistance against pathogen infections in several plants is associated with many biotic and/or abiotic factors, in which they might activate the synthesis of phytoalexins and pathogen-related (PR) proteins such as chitinase and phenylalanine ammonialyase (PAL). Chitinase have the ability to degrade cell wall constituents of fungi (Van Loon *et al.,* 2006; Fornalé *et al.,* 2010). PAL play key role in the phenolic compound production in plant tissues, whereas, it's the first enzyme in the phenylpropanoid pathway (Mandavia *et al.,* 2000; Kim and Hwang, 2014). Peroxidase is potentially associated with host resistance process against the plant diseases including hypersensitivity reaction, lignifications, phytoalexin production, cross-linking of phenolics and glycoproteins (Wojtaszek, 1997; Ippolito *et al.,* 2000; Atia *et al.,* 2005; Almagro *et al.,* 2009).

The present study was designed to evaluate the antifungal activities of some alternative abiotic agents to control barley net blotch disease in which they could help to reduce the amount of fungicides used in crop protection. In addition, to investigate their ability to activate the defense mechanism system in barley plant against net blotch disease.

MATERIALS AND METHODS

Tested Plant Materials

Net blotch highly susceptible commercial barley cultivar Giza 2000 was planted in all experiments under greenhouse conditions at barley Dis. Res. Dep., Plant Pathol. Res. Inst., Agric Res. Center, Giza, Egypt.

Isolation, Purification and Identification of the Causal Organisms

Leaves with typical net blotch symptoms were collected from barley fields distributed across some of barley growing regions from different governorates of Egypt. The causal organism was isolated in water agar medium and pure cultures were obtained using hyphal tip technique (Brown, 1924). Identification of pure culture obtained was carried out at barley Dis. Res. Dep.

Inoculation Technique

Mycelial fragments from 10 days old cultures of *D. teres* grown on PDA plates were obtained by adding 20 ml of distilled water to each plate and homogenized with blender for 5 min. The density of fragment suspension was adjusted to be 10^5 /ml (Badr *et al.,* 2015). Barley leaves were sprayed with water and swabbed with absorbent cotton to facilitate the inoculation of the leaves. Barley plants were inoculated 24 hr., post treatment by spraying the fragment suspension using hand atomizer until run off. The inoculated plants maintained under high relative humidity (100%) at 22±2 °C for 48 hr. Plants were observed daily after inoculation for disease assessment. Disease reaction was recorded 12 days post inoculation (PI). Leaves showed typical symptoms of net blotch (Parry 1990; Liu *et al.,* 2011), were used to re-isolate the causal agent.

Pathogenicity Test

Barley cv. Giza 2000 highly susceptible (provided from Barley Res. Dep., Field Crop Res. Inst., Agric. Res. Center, Giza, Egypt) to *D. teres* was used in pathogenicity test. Disease response was scored by estimating the type of infection of barley net blotch according to Tekauz (1985) scale, rating from highly resistance (0) to very susceptible (10). Disease incidence calculated by the number of infected leaves dividing by total number of examined leaves × 100.

Preparation of Tested Substances and Concentrations

Chitosan (1,4 -2-amino-2-deoxy-β-D glucose) was prepared by dissolving in 0.1% acetic acid under continuous stirring; then the pH was

adjusted to 5.6 using 0.1 M NaOH (Atia *et al.*, 2005). *In vitro* tests, the stock solution (2 g /100 ml) was used to obtain the main tested three concentrations (0.05, 0.1 and 0.15%). Two promising concentrations *i.e.* 0.1 and 0.15% were used *in vivo* trials.

Xanthan gum (a hetero-polysaccharide of high molecular weight) was prepared by dissolving in distilled water and centrifuged two hours before used. *In vitro* trials, as a stock solution (4 g /100 ml) used to obtain 0.1, 0.2 % and 0.3%. Two tested concentrations *i.e.* 0.1 and 0.3% were used *in vivo* trials.

Propolis collected from different areas of Giza (Egypt) was prepared by dissolving 20 g in 70% ethanol in brown glasses and the solution was shaking over night. The resulting ethanol extract was filtered three times through filter paper and three successive concentrations were obtained (0.2, 0.4 and 0.6%). Two promising concentrations *i.e.* 0.1 and 0.15% were used *in vivo* trials (Ozdemir *et al.*, 2010).

Propiconazol fungicide (1-[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl] methyl] -1,2,4-triazole) was used at the recommended concentration (0.25 ml/l) in all *in vitro* and in *vivo* experiments.

In vitro Trials

Effect of tested substances on linear growth of *Drechslera teres*

Three different concentrations of each tested substance (mentioned above) were conducting by adding to warm PDA medium before pouring into 90 mm Petri plates. After solidification, a disk 5 mm in diameter of *D. teres* growth (10 days old culture) was placed in the center of each plate. Untreated plates were used as a control. Three replicates were used for each concentration. Plates were incubated at 20 ± 2 °C. The linear growth was recorded after 10 days. The inhibition percentage in the mycelial growth was calculated using the following formula (Skidmore and Dickenson, 1976):

$$\text{Growth inhibition index (\%)} = \frac{Rc - Rt}{Rc} \times 100$$

Where, Rc is the average radial growth diameter measured in control plates and Rt is the average radial growth diameter measured in treated plates.

Effect of the tested substances on spore germination of *Drechslera teres*

In order to obtain conidia of *D. teres*, leaves with 15 days net blotch developed lesions were incubated in Petri dishes with moist filter papers for 5 days. Under laboratory conditions, the moistened leaves were immersed for 24 hr., in 15 ml test tube containing 7 ml of each tested concentration. A water tubes were used as a control. Number of germinated spores per 100 conidia were counted at several microscopic field x100. The inhibition percentage of the spore germination was calculated using the following formula:

$$\text{Germination inhibition index (\%)} = \frac{Gc - Gt}{Gc} \times 100$$

Where, Gc is the number of germinated conidia in the control and Gt is the germinated conidia in the treatment.

In vivo Trials

Two experiments were conducted under the greenhouse conditions (Plant Pathology Research Inst., Giza) using seedlings and adult plants to evaluate the effect of promising concentrations of chitosan, xanthan gum, propolis and propiconazol on the disease development. Seedlings were grown by planting barley seeds in 10 cm plastic pots (10 plants/ pot). Adult plants were grown under outdoor conditions in 30 cm pottery pots. Barley plants 50 days-old were transferred to the greenhouse. Both seedlings and adult plants were treated with two promising concentrations as mentioned before and control were sprayed with water. Spray was applied until run off. Three replicates were used for each treatment. Twenty four hours post treatment both treated and untreated young (10 days-old) and adult plants (50 days-old) were inoculated as mentioned before.

Disease Incidence Assessment

Disease incidence was assessed by evaluation number and length of lesions 5, 10 and 15 days post inoculation (El-Nashar, 1990).

Enzyme Activity Assays

Leaf samples were collected in ice box from treated and untreated plants for chitinase, Phenylalanine ammonialyase (PAL) and peroxidase assays at 0, 24, 48 and 72 hr., after treatment.

Chitinase activity was assayed according to Rybka *et al.* (1998). For all samples results were expressed as U / mg protein / sec.

Activity of PAL was determined as reported by Lisker *et al.* (1983). For all samples results were expressed as (nano-moles of cinnamic acid / gfw / sec.).

The activity of peroxidase was assayed according to the method of Biles *et al.* (2000). The activity was expressed as the increase in 470 nm absorbance/minute per gram fresh weight.

Statistical Analysis

Data were statistically analysed with Assistat Software, Version 7.7 Beta (Silva and Azevedo, 2009), for analysis of variance (ANOVA) using completely randomized design (CRD).

RESULTS

Isolation, Purification and Identification of the Causal Organisms

Drechslera teres pathogen was individually isolated from typical net blotch naturally infected leaves as well as from artificial inoculated barley leaves. The pathogen was identified according to Luttrell (1951) and Liu *et al.* (2011).

Pathogenicity Test

Typical characteristic symptoms of net blotch introduced with isolated *D. teres* obtained from infected barley leaves. Lesion characterized by dark brown longitudinal streaks with transverse lines, giving a net-like appearance lesions surrounded by areas of chlorosis and large areas of dead tissues can be present. Barley cv. Giza 2000 showed susceptible reaction with the type 10 very susceptible (VS) (Tekauz, 1985). Disease incidence was 93.33% (Table 1 and Fig. 1).

Effects of Tested Substances on Lnear Growth and Spore Germination of *Drechslera teres in vitro*

As shown in Table 2 a significant inhibition of linear growth and spore germination was achieved at all tested concentrations of chitosan (CHT) and propolis (PROP) comparing to control, while xanthan gum (XAN) had no inhibition effect in all tested concentrations. The conidia immersed in the solution of propiconazol and 0.15% CHT showed complete inhibition in spore germination followed by 97.30% in spore germination at 0.1% CHT. Complete inhibition in linear growth was obtained by the propiconazol treatment. No significant differences were observed between CHT concentrations at 0.1 and 0.15 % in which reduced linear growth with 79.44 and 80.63% respectively, while 0.05% CHT showed the lowest effect in reducing both linear growth and spore germination. All tested concentration of PROP significantly reduced the fungal mycelial growth and spore germination. The highest reduction by PROP was obtained with 0.6% (80.77%) of both linear growth and spore germination (69.31%).

Effects of the Tested Treatments on Barley Net Blotch Seedling Plants *In vivo*

Untreated plants showed the highest disease incidence as a number of lesions with the value of 10.22 lesions/leaf. High reduction in the number of lesions with less than two lesions/ leaf was obtained with propiconazol, CHT (0.1 and 0.15%), and XAN at 0.3% treatments. Also, PROP treatment at both tested concentrations revealed a significant difference compared to control (Fig. 2).

The necrotic lesion length was measured at 5, 10 and 15 days PI, and the growth curves are shown in Fig. 3. The lesions consistently grew over time. Data revealed significant differences between tested treatments among all scored times. The lesions length of control plants sharply increased from 11.66 to 43.6 mm from 5 to 15 days PI, followed by PROP (0.4%) with non-significant difference at 10 and 15 days. The most effective concentration on the rate of lesion increase was at propiconazol, 0.1% and 0.15% CHT in which lesion increased from 1.5 to less than 7.5 mm, followed by the application of XAN at 0.3% which revealed high activity against lesion length increased from 3 to 13.5 mm. Plants treated with PROP at 0.6% showed high rate of lesion increase (7.13 to 28.33 mm) with significant difference of un-treated plants.

Table 1. Pathogenicity test of *Derchslera teres* the causal of net blotch disease on barley cv Giza 2000

Pathogen	Type of infection	Disease incidence (%)
Drechslera teres	10 (VS)[a]	93.33
Control	(---)[b]	0.00

[a] (Vs) is very susceptible according to Tekauz (1985) scale. [b] Not infected

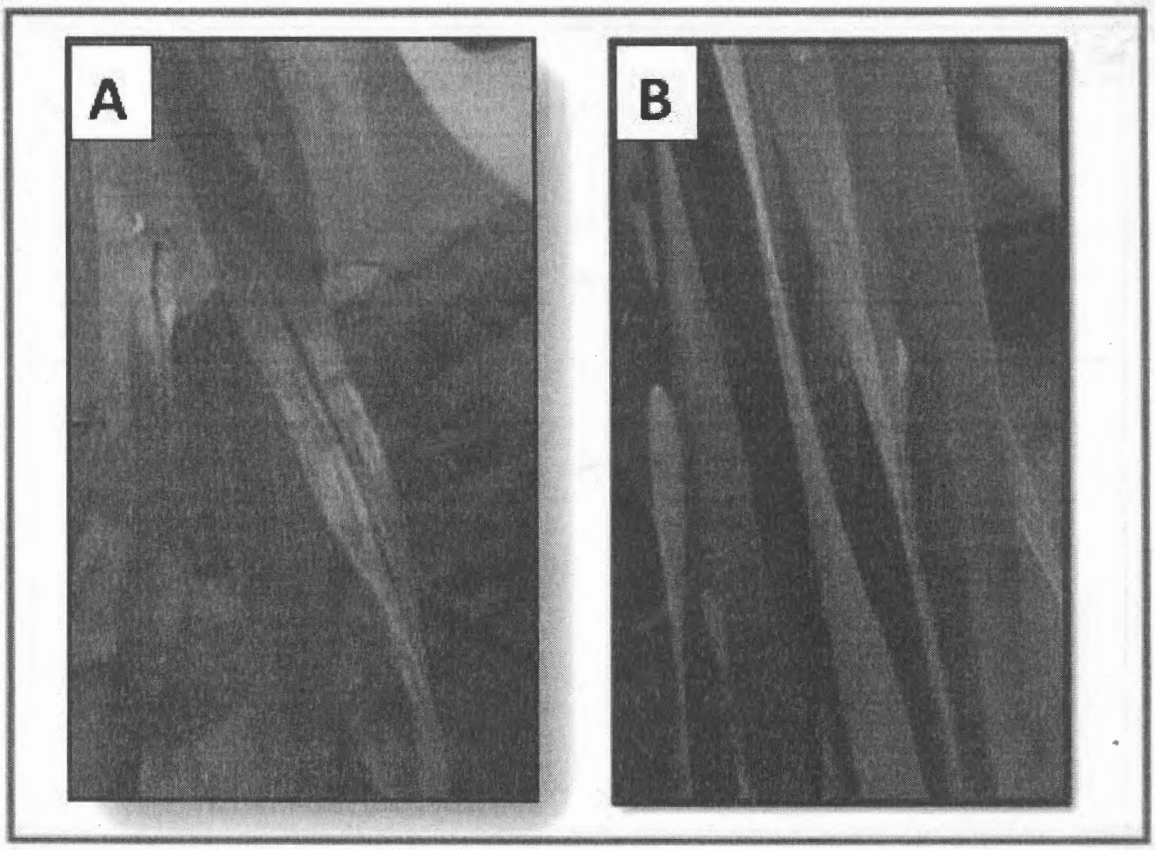

Fig. 1. Typical net blotch symptoms of barley (A) scored 12 days post inoculation with *Dereshslear teres* isolate from infected barley leaves compared to control (B) at seedling stage

Table 2. Effect of tested substances on linear growth and spore germination of *Derchslera teres*

Treatment	Concentration (%)	Linear growth (cm) (Mean ± SE[b])	Reduction (%)	No. of germinated Spores (Mean±SE[b])	Reduction (%)
Control	**S.D.W.[a]**	**9.00 a ± 0.00**		**99.33 a ± 0.34**	–
Chitosan (CHT)	0.05	7.00 b ± 0.54	2.22	24.00 b ± 3.46	75.83
	0.1	1.85 c ± 0.35	79.44	2.60 c ± 0.05	97.30
	0.15	1.76 c ± 0.61	80.63	0.00 c ± 0.00	100
Propiconazol	0.025	**0.00 d ± 0.00**	100	**0.00 c ± 0.00**	100
LSD at 0.05	–	**0.7576**	–	**3.422**	–
Xanthan gum (XAN)	0.1	9.00 a ± 0.00	0.00	100.00 a ± 0.00	0.00
	0.2	9.00 a ± 0.00	0.00	100.00 a ± 0.00	0.00
	0.3	9.00 a ± 0.00	0.00	100.00 a ± 0.00	0.00
Propiconazol	0.025	**0.00 b ± 0.00**	100	**0.00 b ± 0.00**	100
LSD at 0.05	--	--	--	--	--
Propolis (PROP)	0.2	6.83 b ± 0.33	24.11	84.00 b ± 7.38	15.00
	0.4	3.50 c ± 0.13	61.11	47.54 c ± 4.33	52.13
Propiconazol	0.6	1.73 d ± 0.19	80.77	30.66 d ± 3.60	69.31
	0.025	**0.00 e ± 0.00**	100	**0.00 e ± 0.00**	100
LSD at 0.05	–	**0.8324**	–	**9.085**	–

(a) S.D.W : Sterilized distilled water (b) SE : Standard error

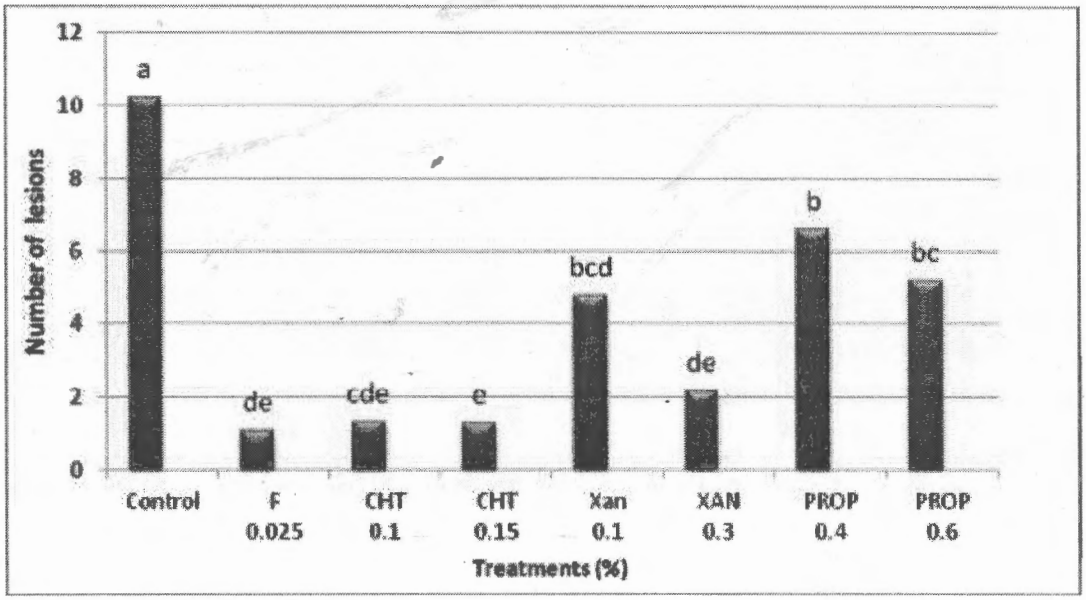

Fig. 2. Number of net blotch lesions scored 5 days post inoculation with *Derchslera teres* on barley seedling plants treated with chitosan (CHT), xanthan gum (XAN), propolis (PROP) and propiconazol (F) (0.25 ml/l) under greenhouse conditions

Effects of the Tested Substances on Net Blotch at Barley Adult Plants *In vivo*

As shown in Fig. 4, all tested treatments significantly reduced the number of leaf spots comparing with untreated adult plants. Tested treatments showed significant different between their concentrations except for CHT. Control plants recorded the highest lesion / leaf (33.62) followed by PROP at 0.4% (26.16) and XAN at 0.1% (24.18). Propiconazol and CHT at (0.1 and 0.15%) revealed the lowest number of lesions/ leaf being 3.68, 6.19 and 4.85, respectively followed by XAN 0.3% and PROP 0.6% (8.50 and 15.17, respectively).

The length of lesions on control plants increased from 18.43 to 44.60 mm at 5 to 15 days post inoculation (Fig. 5). No infection response was recorded on plants treated with propiconazol at 5 days, and then the lesion length grew slowly from 2.07 to 3.53 mm at 10 and 15 days respectively. Both applications of CHT treatments were significantly similar with that recorded in propiconazol treatment at the all assessment times with 2.54 to 3.56 mm for CHT 0.1% and 2.40 to 3.74 mm at CHT 0.15%, followed by the application of XAN 0.3% and PROP 0.6%. Significant differences were obtained between both PROP (0.4 and 0.6%) at 5 and 15 days, in which lesion increased from 11.78 to 21.34 and 7.26 to13.30 mm, respectively (Fig. 6).

Enzymes Activity in Barley Adult Plants Treated with the Tested Substances

Peroxidase (POD) activity was very higher with propiconazol, followed by XAN 0.3%, PROP 0.6% and CHT (0.1 and 0.15%). A greater increase in POD activity was measured 24, 48 and 72 hr., after treatment with propiconazol (4.4, 3.6 and 3.2-fold increase, respectively) compared to the control (0.4, 0.7 and 0.6-fold, respectively). Leaves treated with CHT 0.1% showed the peak of activity after 24 hr., (0.9-fold), reaching the maximum level at 72 hr., (1.6-fold), before declining at 48 hr., (0.64-fold).

Application of XAN at 0.3% showed higher increase in POD activity reaching its maximum after 48-72 hr., (2.3 to 3.5-fold), while leaves treated with 0.1% revealed low activity reaching its maximum after 72 hr., (0.9-fold). Application of PROP at 0.6% resulted in an increase of POD activity reaching a maximum at 48 (2.6 fold), while the peak of activity for 0.4% PROP reaching a maximum at 24 (0.8-fold) then sharply decreased (0.3-fold) at 48 hr., and increased with 0.7-fold at 72 hr., (Fig. 7).

Chitinase (CHS) activity showed the highest stimulation response with PROP 0.6%, CHT at 0.1 and 0.15%, followed by XAN 0.3%, while the lowest response was observed by propiconazol application (Fig. 8). The application of CHT (0.1%) reaching their maximum at 24 and 72 hr., (1.3 and 2.2-fold) before decreasing at 48 hr., (0.9-fold). The peak of activity at the application of CHT at 0.15% was increased with 1.1, 1.9 and a 1.04-fold at 24, 48 and 72 hr., respectively. CHS activity in XAN at 0.3% treated plants increased steadily at 24 - 48 hr., (0.7-0.8 fold) then reaching its maximum at 72 hr., (1.3-fold), while low increase in CHS activity was observed in leaves treated with XAN 0.1% with 0.39, 0.58 and 0.3 fold at 24, 48 and 72 hr., respectively. The application of PROP at 0.6% resulted in increased activity at all tested times to reaching maximum at 48 hr., with 2.1 fold, whereas, reaching its maximum in leaves treated with PROP at 0.4% was at 24 hr., (1.1-fold), in which decreasing henceforward to reach 0.8 and 0.6-fold at 48 and 72 hr., respectively.

The higher activity of phenylalanine ammonialyase (PAL) was observed in plants treated with propiconazol followed by CHT 0.1 and 0.15% (Fig. 9). Plants treated with propiconazol showed very high response at 24 hr., where activities reaching its maximum (5.8-fold) PAL. Treatments with PROP showed the lowest activities among all tested treatments with non significant difference compared with untreated plants. Activities of PAL reached their maximum at 24 hr., for PROP (0.4 and 0.6%) with 0.56 and 0.3-fold, respectively.

DISCUSSION

Net blotch disease attacks most of the Egyptian barley commercial varieties (El-Nashar, 2000; El-Nashar *et al.,* 2008; Badr *et al.,* 2015). Pathogenictiy test of the current work revealed virulent isolate of *Drechslera teres* (*D. teres*) in which produced highly susceptible reaction on barley cv. Giza 2000. Results are

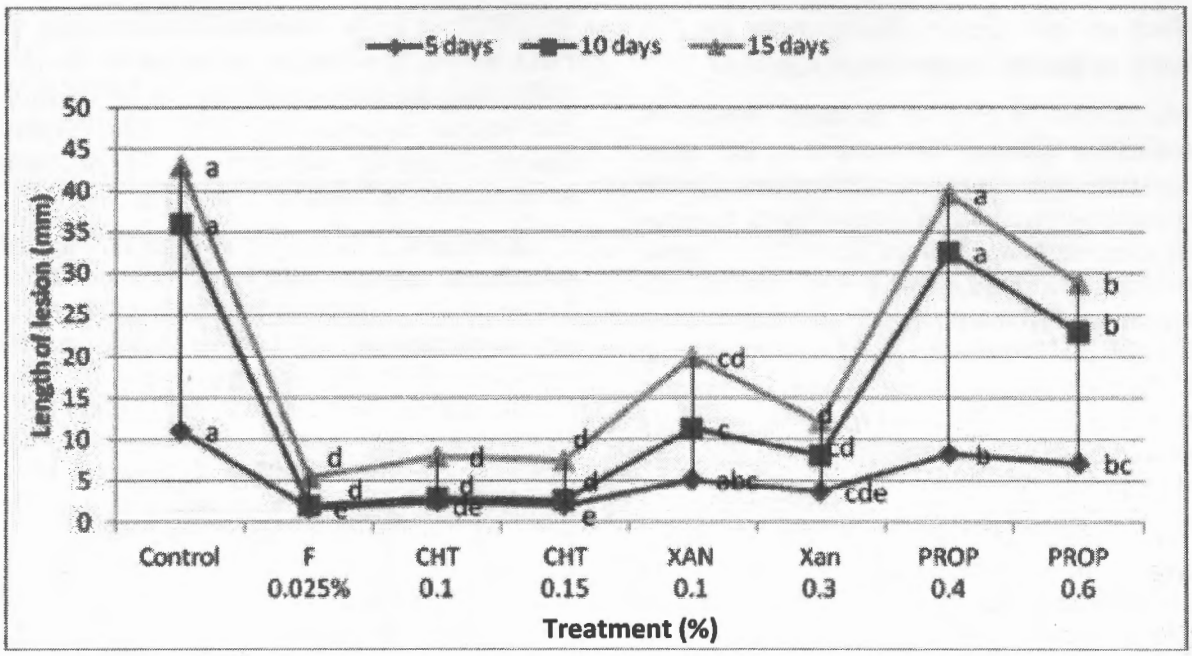

Fig. 3. Length of net blotch lesion scored 5, 10 and 15 days post inoculation with *Derchslera teres* on barley seedling plants treated with chitosan (CHT), xanthan gum (XAN), propolis (PROP) and propiconazol (F) (0.25 ml/l) under greenhouse conditions

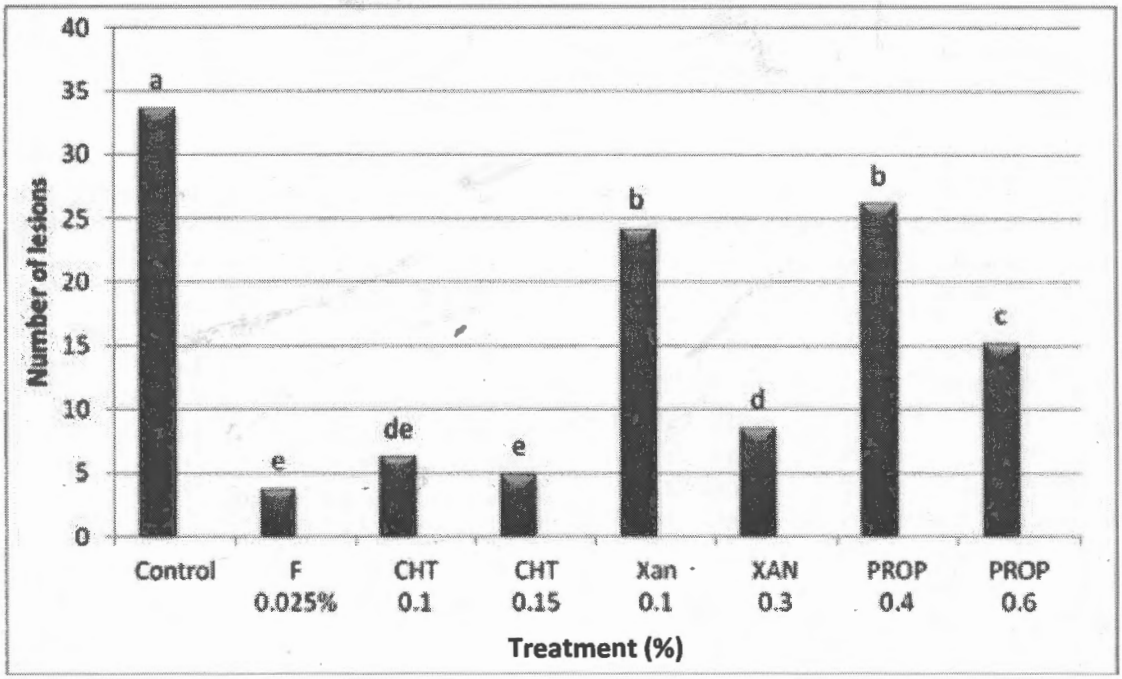

Fig. 4. Number of net blotch lesions scored 5 days post inoculation with *Derchslera teres* on barley adult plants treated with chitosan (CHT), xanthan gum (XAN), propolis (PROP) and propiconazol (F) (0.25 ml/l) under greenhouse conditions

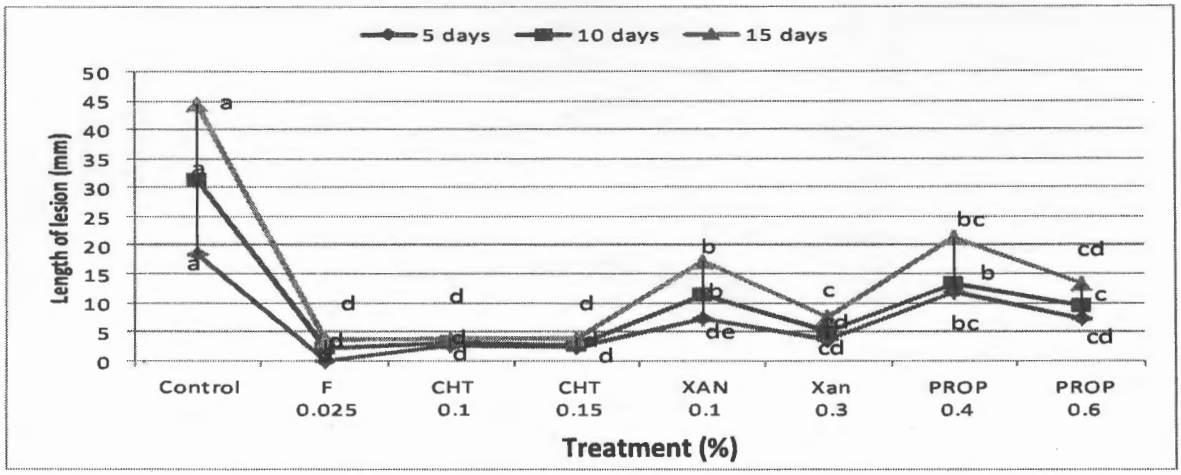

Fig. 5. Length of net blotch lesion scored 5, 10 and 15 days post inoculation with *Derchslera teres* on barley adult plants treated with chitosan (CHT), xanthan gum (XAN), propolis (PROP) and propiconazol (F) (0.25 ml/l) under greenhouse conditions

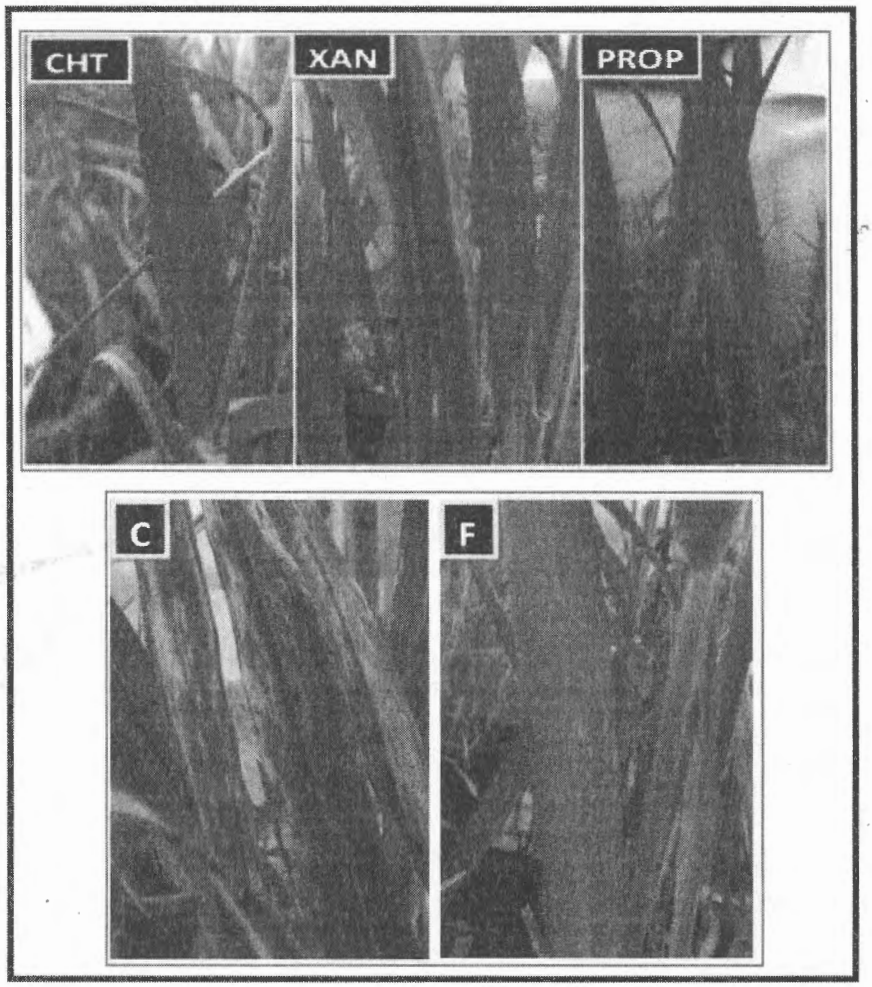

Fig. 6. Net blotch response scored 10 days post-inoculation with *Derchslera teres* under greenhouse conditions on adult barley plants treated with chitosan (CHT) at 0.1, xanthan gum (XAN) at 0.3%, propolis (PROP) and propiconazol (F) (0.025%) comparing with control (C)

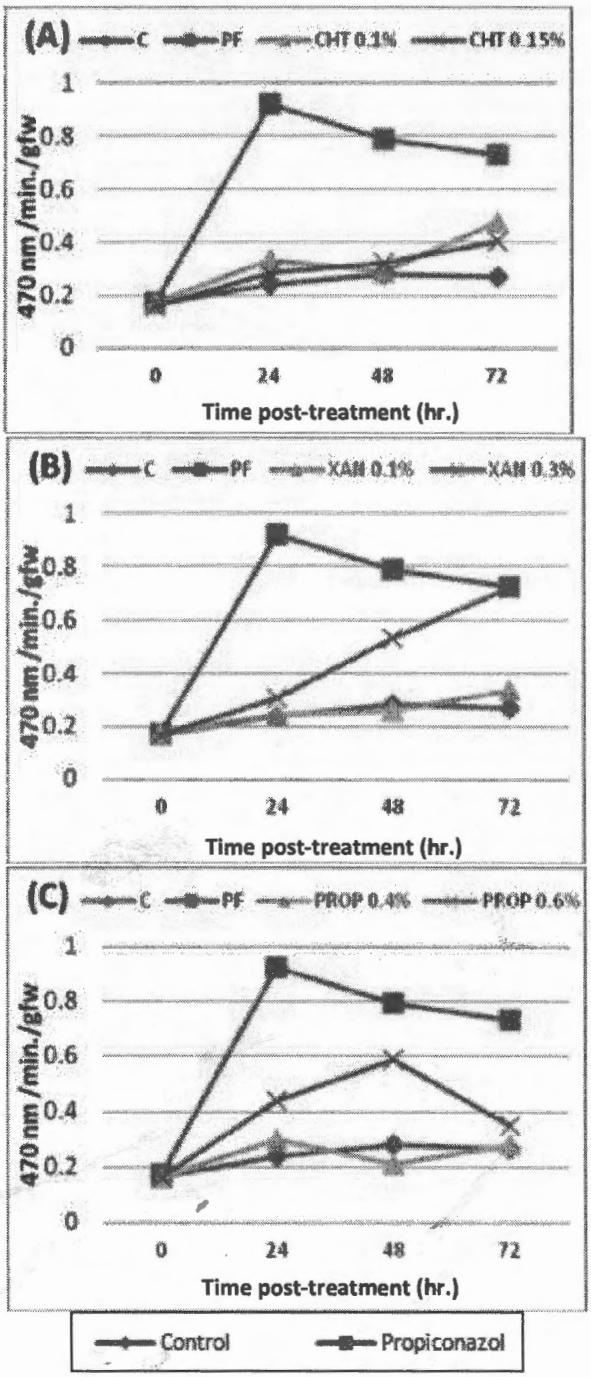

Fig. 7. Peroxidase (POD) activity in barley adult plants treated with chitosan (A), xanthan gum (B), propolis (C) and propiconazol (PF) (0.25 ml/l)

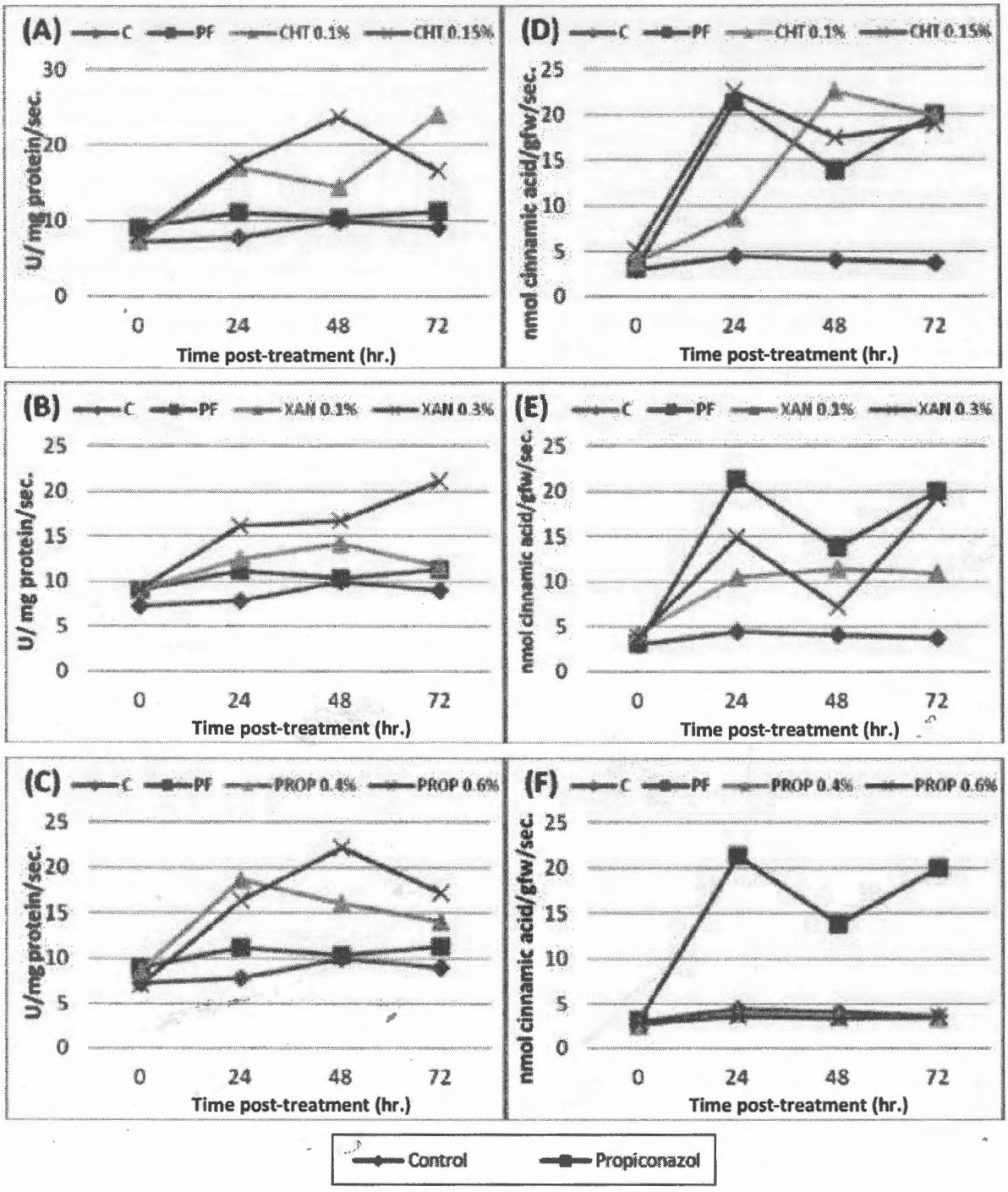

Fig. 8. Chitinase (CHS) activity in barley adult plants treated with chitosan (A), xanthan gum (B), propolis (C) and propiconazol (PF) (0.25 ml/l)

Fig. 9. Phenylalanine ammonialyase (PAL) activity in barley adult plants treated with chitosan (D), xanthan gum (E), propolis (F) and propiconazol (PF) (0.25 ml/l)

quite similar with other previous studies. Badr *et al.* (2015) evaluated the net blotch reaction of 12 pathotypes of *D. teres* isolated from different areas of Egypt on 14 Egyptian cultivars. Barley cv. Giza 2000 showed the highest susceptible reaction and was infected by all tested pathotypes. Also, previous studies carried by El-Nashar (2000) and El-Nashar *et al.* (2008) strongly support the current finding.

The present study was planning to investigate and maintain prospective alternatives for the control of barley net blotch disease , testing the direct and indirect effect of some abiotic agent such as chitosan (CHT), xanthan gum (XAN), propolis (PROP) against barley net blotch compared with the propiconazol fungicide at the recommended dose (0.25m/l).

The *in vitro* results approved the effectiveness of tested alternative materials in reducing growth and spore germination of *D. teres*, the causal organism of barley net blotch. Chitosan showed a high inhibitory effect on spore germination than mycelial growth at the same concentrations. These findings are in the same line with Bhattacharya (2013) who found that, spore germination of *Fusarium solani* were completely inhibited with CHT 0.2% whereas the fungal linear growth inhibited by 76 % at the same concentration. Also, several reports are in the same trend with the present findings (Hernández-Lauzardo *et al.,* 2008; Palma-Guerrero *et al.,* 2008; Pabón-Baquero *et al.,* 2015; El Guilli *et al.,* 2016). Opposite results were found by Meng *et al.* (2008), they reported that, both chitosan and oligochitosan strongly inhibited mycelial growth more than spore germination of *Alternaria kikuchiana* and *Physalospora piricola.* Generally, the *in vitro* fungicidal activity of chitosan against mycelial growth and spore germination has been well documented (Atia *et al.,* 2005; Guerra-Sánchez *et al.,* 2009; Rabea *et al.,* 2009). Previous studies has been proposed the inhibitory effect of chitosan on phytopathogenic fungi can be obtained by neutralized the plasma membrane of fungal cell result in membrane destabilization (Chio *et al.,* 2001), and/or by the penetration of the cell wall causes intracellular disruption (Guo *et al.,* 2008; Palma-Guerrero *et al.,* 2008). In addition, it can cause changes in the membrane integrity of spores, modifications in pH media and the proteins release (Hernández-Lauzardo *et al.,* 2012).

Xanthan gum *in vitro* hadn't any fungicidal or fungistatic activities on mycelial growth and spore germination of *D. teres*. Generally, xanthan gum is nontoxic substance and doesn't inhibit growth and has been used in a wide variety of foods for a number of important reasons, *i.e.* emulsion stabilization and compatibility with food ingredients (Garcoaa-Ochoa *et al.,* 2000). A trial conducted by Arismendi *et al.* (2013) illustrated that, XAN as food application supporting potassium sorbate with the role of controlling the external food contamination with molds by formation an edible film resulted in an effective antimicrobial barrier. While, addition of XAN alone increased molds. In addition, XAN food solutions were unable to protect the antimicrobial activity of preservative ingredients against the spoilage microbes (Si *et al.*, 2006).

The fungicidal activity of propolis within this study against mycelial growth and conidial germination of *D. teres* revealed different range of sensitivity at the tested concentrations. Conidial germination showed a low sensitivity compared to mycelia growth in which reach their maximum inhibition (69.31%) at 0.6% PROP. Several studies regarding antimicrobial avtivity assays of propolis against many plant pathogenic fungi have been literatured (Valencia *et al.*, 2012). Flavonoids and phenolics are proposed to be the main antimicrobial constitution of propolis particularly towards phytopathogenic fungi (Treutter, 2006; Yang *et al.*, 2011). The mode of action of flavonoids and phenolics are mainly by the penetration of the microorganisms causing considerable damage to the cell metabolisms by the crosslinking of enzymes and inhibition of some fungal enzymes (Shukla and Dwivedi, 2013).

In vivo experiments under greenhouse condetions, the lesions were increased with a rate much high in seedlings than in the case of adult plants treated with the same concentrations of tested substances. This might be genetically suggested as the base of defense resistance mechanism in which Steffenson *et al.* (1996) reported that only two or three loci were found to confer resistance to the net blotch pathogen at

the seedling stage, whereas seven loci were identified for net blotch resistance at the adult plant stage. Results of the present study illustrated a various level of protection against net blotch with both seedlings and adult plants treated with the tested substances. Chitosan was the most effective one with no significant difference compared to the propiconazol fungicide at its recommended concentrations in all disease components. The present results are consistent with previous studies that have shown that chitosan can deter a variety of fungal plant diseases (Atia *et al.,* 2005; Nandeeshkumar *et al.* 2008; Romanazzi *et al.,* 2009). Liu *et al.* (2012) illustrated that, foliar application of chitosan reduce the disease incidence of rice seedlings inoculated with *Rhizoctonia solani.* Chitosan found to mediate physiological changes by the induction of resistance during the accumulation of such enzymes involved in plant defense mechanism. (Atia *et al.,* 2005; Katiyar *et al.,* 2015). The present study demonstrated their ability to trigger plant defense mechanism to barley net blotch disease by increase the activity of chitinase CHS, POD and PAL. These results are in agreement with several works on defferent crops (Atia *et al.,* 2005; Agrawal *et al.,* 2002; Yin *et al,* 2008; Chen *et al.,* 2016). A trial conducted by Sathiyabama *et al.* (2014) reported that, tomato leaves treated with chitosan induced a high level of chitinase activity resulting in the reduction of early blight disease severity. Khan *et al.* (2003) stated that, soybean treated with chitosan increased activities of PAL and tyrosine ammoina–lyase (TAL) in leaf tissues resulting in an increase in the level of protection against plant pathogen. Increase of POD activity in barley plants treated with CHT was relatively low compared to the CHS and PAL and this are on line with Guo *et al.* (2003) who found that, POD activity in wheat leaves treated with CHT slightly increased with 0.0625 and 0.125 times more than control.

The present data also showed that XAN application showed a high level of protection against net blotch disease in both adult and seedling stages. Leaves treated with XAN showed a high restriction on lesion increase with the high level of protection in leaves treated with 0.3%. Few previous findings showed the ability of XAN to protect plants from pathogen attack. Leaves treated with XAN presented 92% of protection in coffee plants against rust disease (Guzzo *et al.,* 1993). In the present study, a low level of infection response in leaves treated with XAN is an indication of their ability to trigger plant defense booster against net blotch of barley. The development of resistance has been in response to the increase of enzymes activities. XAN application increased all analyzed enzymes activity over the control. Few reports are in line with the present findings, in which indicated that the application of XAN suppress the development of the plant pathogen due to the induction of both local and systemic resistance mechanism resulting in accumulation of PR-proteins leading to protection greater than 90% (Bach *et al.,* 2003; Castro and Bach 2004). The mode of action of XAN to protect the plant from the pathogen infection has not been clearly understood. It may be attributable to the glucose structure unit of the natural polysaccharides, in which many investigations showed that glucose unite is an essential signaling molecules that could play important role in regulation of gene expression of plant enzymes (Morkunas *et al.,* 2005; Hofmann *et al.,* 2010) and their ability to form a physical barriers (Ippolito *et al.,* 1997).

The propolis treatment of the present findings have proven effective action compared to control net blotch disease components but almost, it showed the lower level of protection, which might be due to the ability of some fungi to develop counter-defense mechanisms against the flavonoids during the detoxification and / or metabolization of this antimicrobial by fungal extracellular enzymes (Medina *et al.,* 2004; Pedras and Ahiahonu, 2005). This study state that propolis application induced both CHS and POD activities over the control, while PAL enzyme activity didn't increased with PROP application. Such results are in agreement with Mahdy *et al.* (2006). In conclusion, chtiosan, xanthan gum and propolis alternative substances have the ability to manage barley net blotch disease under greenhouse conditions with varying level of protection, but more further studies should be taken to evaluate this tested substances under field conditions in which could open avenues for the approach of reducing number of synthetic fungicides chemicals applied to crops.

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قـــدرة بعـــض المركبـــات غيـــر الحيـــويـــة لمكـــافحـــة مرض التبقـــع الشـــبكي في الشـــعير

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تم دراسة تأثير بعض المركبات غير الحيوية مثل الشيتوزان وصمغ الزانثان والبروبوليز مقارنة بالمبيد الفطرى بروباكونازول بمعدل ٠,٢٥ مل/لتر (الجرعة الموصى بها) ضد مرض التبقع الشبكى فى الشعير المتسبب عن الفطر *Drechslera teres* تحت ظروف المعمل والصوبة، أظهرت التجارب المعمليه تأثير عالى لمعامله الشيتوزان على تثبيط انبات الجراثيم عنه فى حالة النمو الخطى للفطر بينما حدث عكس ذلك فى حالة مادة البروبوليز، ولم يظهر أي تأثير مثبط لمعاملة صمغ الزنثان على كل من إنبات الجراثيم والنمو الميسليومى للفطر، أظهرت جميع المعاملات تحت ظروف الصوبه تأثيراً معنوياً في مكافحة المرض بدرجات متباينة مقارنة بالنباتات غير المعاملة، حيث أظهرت المعاملة بالشيتوزان والبروباكونازول أعلى تأثير مثبط للمرض على كل من البادرات والنباتات البالغة، وذلك عن طريق عدد ومعدل زيادة طول البقع الناتجه عن الإصابة بالفطر المسبب، وقد تلا ذلك المعاملة بماده صمغ الزنثان، أظهرت المعاملة بالبروبوليز التأثير الوقائى الاقل بين المعاملات لا سيما على طور البادرة، وتم تقييم قدره هذه المركبات على تعزيز وسائل الدفاع للنبات عن طريق تقدير نشاط بعض الإنزيمات المسئولة عن المقاومة مثل نشاط انزيم البيروكسيديز والشتينيز والفنيل الانين امونيا لايز، وقد أظهرت النباتات المعاملة بالبروباكونازول النشاط الاعلى لانزيم البيروكسديز تليها المعاملة بالبربوليز (٠,٦%) وصمغ الزنثان (٠,٣%)، أظهر نشاط إنزيم الشتينيز إستجابه عاليه مع كل من معاملة البربوليز (٠,٦%) والشيتوزان (٠,١ و ٠,١٥%)، أظهرت المعاملة بالبروباكونازول والشيتوزان النشاط الأعلى لإنزيم الفنيل الانين اميونيا لايز.

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