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Application of plant extracts as inducers to challenge leaf rust of wheat

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Abstract

Five plant extracts, i.e., henna, *Lawsonia inermis*; acalypha, *Acalypha wilkesiana*; chinaberry, *Melia azedarach*; pomegranate, *Punica granatum*; and lantana, *Lantana camara*, were tested as inducers to protect wheat against leaf rust infection caused by *Puccinia triticina* Eriks. The plant extracts were applied pre-infection on susceptible wheat cultivar "Gemmiza-7" under field conditions during two growing seasons (2016/2017 and 2017/2018). All the tested plant extracts were found to be effective against the leaf rust infection. They significantly reduced the coefficient of infection (ACI) to be ranging 7.50 to 20.00, compared to the non-treated control (ACI = 75.00). Lantana extract was the most effective one (efficiency = 88.88%), which was very close to the fungicide "diniconazole" (efficiency = 89.92%). Henna extract ranked second (80.00%), followed by chinaberry (76.00%), acalypha (72.00%), and pomegranate (68.00%). However, wheat yield components were significantly increased by all the tested treatments, especially lantana extract and the fungicide. Similarly, biochemical analyses revealed a significant increase in the plant contents of chlorophyll a and b, total phenolics, and oxidative enzymes activities (POX and PPO) at all the tested treatments. Results indicated that the tested plant extracts could induce wheat resistance to leaf rust.

Keywords: Wheat leaf rust, *Puccinia triticina*, Induced resistance, Plant extracts, *Triticum aestivum*

Background

Leaf rust caused by *Puccinia triticina* Eriks is one of the most serious constraints in wheat production in many wheat-growing regions. In Egypt, the fungus causes a severe yield loss reached up to 50% (Abdel-Hak et al. 1980 and Draz et al. 2015). Disease management is usually based on the use of resistant cultivars (Draz et al. 2015) and application of synthetic fungicides (Barro et al. 2017). The leaf rust fungus can form new races that are capable to breakdown the plant resistance. Besides, the negative environmental impacts of fungicides are intensively increasing every day. Thus, the alternative methods for reducing fungicides' use are being developed including plant extracts, as one of the effective methods that incorporate natural antifungal substances. Some plants contain certain components that are toxic to plant pathogens, namely, botanical pesticides or botanicals (Dubey et al. 2008). In fact, natural products have proved to be potential sources of environmentally safe antimicrobial agents, which could be useful in plant protection and plant disease control (Wang et al. 2004).

Resistance can be systemically induced in some susceptible plants by the application of certain chemical substances as well as the pre-inoculation with pathogenic or nonpathogenic microorganisms (Kuc 1982). In this subject, botanical extracts have been found to effectively control a wide range of plant pathogens through inducing a defense response in the infected plants (Chakraborty and Chakraborty 2010 and Srivastava et al. 2011). Studies on the antifungal activity of botanical extracts to protect plants from diseases have received much attention (Morsy et al. 2011 and Bhuvaneshwari et al. 2015). The mode of action of abiotic inducers against plant pathogens might occur as a secondary messenger enhancing the host defense mechanisms (Geetha and Shetty 2002), either by increasing the activity of peroxidase (POX), by the synthesis of new peroxidase isozymes (POD) isoforms, by the accumulation of the phenolic compounds (Hassan et al. 2007), or through inhibition

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of some antioxidant enzymes and catalases, thereby leading to the production of elevated amounts of H_2O_2 (Radwan et al. 2008). In addition, abiotic inducers also enhance resistance through direct effects on the development and survival of the pathogens or indirect effects on plant metabolism with subsequent effects on the pathogen-food supply (Khan et al. 2003).

The aim of this study was to investigate the prior applications of some plant extracts for inducing resistance of wheat against leaf rust caused by *P. triticina* under field conditions.

Materials and methods

Plant materials

Plant extracts of five plant species, including leaves of henna (*Lowsonia inermis*), acalypha (*Acalypha wilkesiana*), chinaberry (*Melia azedarach*), and lantana (*Lantana camara*) and fruit peel of pomegranate (*Punica granatum*) were tested to determine their efficiency as inducer materials to resist leaf rust infection of wheat under Egyptian field conditions. Fresh and healthy leaves of lantana, acalypha, and chinaberry were collected from the Experimental Farm of Gemmeiza Agricultural Research Station, Agricultural Research Center (ARC), Egypt. Pomegranate and henna were obtained from the local market. Plant samples were washed by tap water to remove dust, then dried in refresh air for 4 days. Dried samples were kept in a refrigerator at 4 °C till use.

Preparation of plant extracts

The plant extracts were prepared according to the method described by Hussain et al. (2012). In which, the used part of plants was grinded individually to semi-powder, using a grinder (LG BL 999SP). Briefly, 10 g of ground sample was extracted by 100 ml of sterilized distilled water in a conical flask and kept for 8 h at room temperature. The filtrate was separated from the solid residue by filtering through Whatman no. 1 filter paper. The process was repeated three times, and the obtained extracts were pooled. A stock solution of each extract was kept in the refrigerator at − 4 °C until use.

Application process

The experiment was carried out at the Experimental Farm of Gemmeiza Agricultural Research Station, ARC, Egypt, during two growing seasons (2016/2017 and 2017/2018). Grains of the susceptible wheat cv. "Gemmeiza-7" were sown in random plots ($3 \times 2\ m^2$) at the rate of 40 g/plot. The experiment was laid out in a randomized complete block design (RCBD) with 3 replicates. The plant extracts were prepared at concentrations of (10,000 mg/l) and applied on leaves, using a hand sprayer until wetness of plants. The chemical fungicide, Fungshow (diniconazole) at 0.15 g/l, served as a comparable control. The plants were sprayed at booting plant growth stage, prior to the artificial inoculation of plants, by urediniospores powder mixture of *P. triticina* isolates (one volume of fresh urediniospores, 20 volume of talcum powder) according to Tervet and Cassel (1951). The plants were moisturized by a fine spray with water then dusted with urediniospores powder mixture of *P. triticina* isolates. Dusting was carried out at sunset before dew onset. Inoculation was done during the second half of February at the 7–8th growth stages adopted by Large (1954). The non-treated control plots were sprayed with distilled water.

Disease assessment was done based on the infection types of wheat leaf rust according to Johnston and Browder (1966), where immune (0) = no uredia or other macroscopic sign of infection, resistant (R) = small uredia surrounded by necrosis, moderately resistant (MR) = small to medium uredia surrounded by chlorosis or necrosis, moderately susceptible (MS) = medium-sized uredia that may be associated with chlorosis, and susceptible (S) = large uredia without chlorosis or necrosis. Disease severity was expressed as the percentage coverage of leaves as described by Peterson et al. (1948). The average coefficient of infection (ACI) was calculated according to Saari and Wilcoxson (1974) by multiplying of disease severity and constant values of infection types. The constant values for infection types were used based on the following: R = 0.2, MR = 0.4, MRMS = 0.6, MS = 0.8, and S = 1.0.

The efficacy of a certain treatment was determined according to the following equation adopted by Rewal and Jhooty (1985):

$$\text{Efficiency}\ (\%) = \frac{C - T}{C} \times 100$$

where C = infection in the control and T = infection in the treatment.

At harvest, the effects of treatments on grain yield components in terms of spike weight, 1000-kernel weight, and volume weight were estimated.

Biochemical assay

Biochemical changes in the wheat leaves treated with plant extracts were estimated at the Laboratory of Department of Agricultural Botany, Faculty of Agriculture, Tanta University, Tanta, Egypt. Leaf samples representing each treatment and controls were collected from plants at the 1st, 3rd, and 15th days after spraying to determine their effects on some of the biochemical components such as chlorophyll, phenols, and oxidative enzymes.

Estimation of chlorophyll contents

The contents of the total chlorophylls (a + b) were determined according to Dere et al. (1998). Fresh leaves (0.1 g) were cut into small fragments (1 × 1 mm) and immersed for 24 h at 4 °C in 20 ml methanol (96%) and then filtered through Whatman 47 mm GF/C filter paper. The absorbance of each filtrate was measured against a blank of 96% methanol at wavelengths of 666 and 653 nm for chlorophyll a and b, respectively. Results were expressed as mg g^{-1} fresh weight and calculated, using the following formulas (Lichtenthaler and Wellburn 1983):

Chlorophyll (Chl.) a = (15.65 A_{666} − 7.34 A_{653})
Chlorophyll (Chl.) b = (27.05 A_{653} − 11.21 A_{666})
Total chlorophyll = Chl. a + Chl. b

Estimation of phenol content

Total phenol content was estimated according to the method described by Malik and Singh (1980); 0.5 g of fresh leaves was ground by 10 ml of 80% ethanol and stored in a dark bottle at 4 °C for 72 h. Extracts were combined and filtered for determination, using Unico UV-2100 Spectrophotometer. The total phenol was measured by Folin-Ciocalteu's reagent, and the absorbance was read at 650 nm. The total phenol was expressed in mg g^{-1} fresh weight.

Estimation of oxidative enzyme activity

Peroxidase (POX) activity was directly determined according to a typical procedure proposed by Hammerschmidt et al. (1982). Polyphenoloxidase (PPO) activity was determined according to the method described by Malik and Singh (1980), in which, 0.5 g leaf material was homogenized at 0–4 °C in 3 ml of 50 Mm TRIS buffer (PH 7.8), containing 1 mM EDTA-Na2 and 7.5% polyvinylpyrrolidone. The homogenates were centrifuged (12,000 rpm, 20 min, 4 °C), and the total soluble enzyme activities were measured spectrophotometrically in the supernatant. Measurements were carried out at 25 °C, using a spectrophotometer (model UV-160A, Shimadzu, Japan). The enzyme assays were tested three times. Changes in absorbance at 470 nm for POX activity and 495 nm for PPO activity were recorded at 30-s intervals for 3 min. Enzyme activity was expressed as the increase in absorbance min^{-1} g^{-1} fresh weight.

Statistical analysis

Obtained data were subjected to the analysis of variance (ANOVA), using the Statistical Analysis System package SAS software v.9.2 (SAS Institute 2010). Means were separated, using the least significant difference (LSD) test at $P \leq 0.05$ (Steel and Torrie 1980).

Results and discussion

Effect of plant extracts on wheat leaf rust disease severity

Data presented in Table 1 show that pre-infection spraying of wheat plants (Gemmeiza-7) with the 5 evaluated plant extracts had an effective role against leaf rust infection. The disease incidences, expressed as the average coefficient of infection (ACI), were significantly reduced by the plant extracts to 7.5 (lantana), which was insignificantly different than the fungicide treatment (ACI = 6.3). However, the ACI value for the non-treated control reached up to (75.00). All the tested plant extracts were very effective in reducing the ACI values on the infected wheat plants where the efficiency of these extracts ranged between 68% for pomegranate peels and 88% for lantana leaves (Table 1).

The findings showed that the prior application of the plant extracts of henna, acalypha, chinaberry, pomegranate, and lantana were good defense inducers for protecting wheat plants from the disease *P. triticina*, under field conditions. Similar results were found by Shabana et al. (2017) who reported a significant reduction in the leaf rust infection of wheat plants, using some plant extracts (garlic, clove, garden quinine, Brazilian pepper, anthi mandhaari, black cumin, white cedar, and neem), sprayed pre-infection on wheat seedlings. In vivo assay, methanol extract of *Curcuma zedoaria* rhizomes exhibited a strong activity against *P. triticina*. When the *C. zedoaria* methanol extracts were partitioned by various solvents, the layers of nhexane, methylene chloride, and ethyl acetate showed disease control values of (100, 80, and 43%), respectively (Han et al. 2017). Kumar et al. (2017) reported that the foliar spray of potato plants with *Lantana camara* extract, as an inducer before the inoculation with *Alternaria solani*, led to decrease the disease severity. Foliar spraying of plant extracts (pomegranate, eucalyptus, cactus, garlic and neem) significantly decreased leaf rust severity of wheat (Abd El-Malik and Abbas 2017). Antifungal activity of volatile components extracted from stem, leaf, and flower extracts, prepared from *L. camara*, showed a strong inhibitory effect against *A. solani*, *Botrytis cinerea*, *Fusarium solani* f. sp. *cucurbitae*, *F. oxysporum* f. sp. *niveum*, *Pythium ultimum*, *Rhizoctonia solani*, and *Verticillium dahlia* (Boughalleb et al. 2005). Neem oil was found to give a significant protection against rust and downy mildew of alfalfa when applied as plant spraying (Morsy et al. 2011).

Data in Table 1 revealed that all the tested plant extracts significantly increased yield components of wheat plants infected with *P. triticina* in terms of spike weight, 1000-kernel weight, and volume weight (L), compared to the non-treated control. Lantana and henna extracts gave the highest spike weight (3.83 and 3.43 g, respectively) and 1000-kernel weight (46 and

Table 1 Effect of the tested plant extracts on the average coefficients of infection (ACI) of wheat (cv. Gemmeiza-7) leaves by the leaf rust fungus, *Puccinia triticina*, and on the wheat yield components, under field conditions

Treatment	ACI	Efficiency (%)	Yield components		
			Spike weight (g)	1000-kernel weight (g)	Volume weight/L
Henna	12.50 cd	80.00	3.55 b	43.24 b	718.10 b
Acalypha	17.50 c	72.00	3.44 b	40.23 c	705.84 d
Chinaberry	15.00 cd	76.00	3.54 b	42.63 b	711.25 c
Pomegranate	20.00 c	68.00	3.43 b	39.50 c	705.10 d
Lantana	7.50 de	88.00	3.83 a	46.00 a	724.30 a
Fungicide	6.30 e	89.92	3.55 b	44.50 ab	711.50 c
Control	75.00 a	–	2.85 d	35.03 d	639.80 f
LSD 0.05	7.86	–	0.20	1.89	2.20

Data are average of three replicates
Means followed by the same letter(s) are not significantly different according to $LSD_{0.05}$

43.24 g, respectively). However, acalypha and pomegranate gave the lowest values of these parameters. The highest volume weight (L) was also obtained by lantana extract (724.30 g), followed by henna (718.10 g), chinaberry (711.25 g), acalypha (705.84 g), and pomegranate (705.10 g). Lantana and henna were the best treatments in increasing the yield parameters of the *P. triticina*-infected wheat plants and superimposed the fungicide "Fungshow" in this respect (Table 1). These results show that yield components of wheat could be improved through treating by the plant extracts tested in this study, that increase spike weight, 1000-kernel weight, and volume weight. Obtained findings are in agreement with those reported by Shabana et al. (2017) who reported that the 1000-kernel weight of leaf rust-infected wheat sprayed with Brazilian pepper extract was improved by 15.73% than the untreated control, followed by white cedar (13.81%) and garlic (13.02%). They also found that the test weight increased by 4.48% when treated with garden quinine extract, followed by garlic (4.13%) compared to untreated. Abd El-Malik and Abbas (2017) reported that the foliar spraying of plant extracts (pomegranate, eucalyptus, cactus, garlic, and neem) significantly increased wheat yield components, including 1000-kernel weight and spike weight.

Biochemical analysis of wheat leaves treated with plant extracts

Chlorophyll content

Data illustrated in Fig. 1 show that the total chlorophyll contents was increased gradually up to 15th day after spraying with the tested plant extracts than in the non-treated control, which showed noticeable decrease, especially after the same period (15 days). Generally, all the tested plant extracts significantly increased the total chlorophyll contents (a + b) in wheat leaves infected with *P. triticina*, compared to the non-treated control. At the first day after spraying with plant extracts, the most effective treatment in increasing the total chlorophyll was henna extract (3.69 mg chlorophyll/g fresh weight), followed by lantana (3.37 mg/g). However, on the 3rd and 15th days, lantana extract was the most effective (3.95 and 4.39 mg/g, respectively), followed by henna extract (3.80 and 4.00 mg/g), compared to the non-treated control (2.53 and 1.69 mg/g) and the fungicide treatment (2.99 and 3.43 mg/g).

Induced resistance is environmentally friendly and confers to long-lasting protection against a broad spectrum of plant pathogens, diseases, bacteria, fungi, oomycetes, and nematode (Durrant and Dong 2004). Induced resistance is generally characterized by the increased synthesis of the chemical compounds in plant that can prevent pathogen's growth and development, due to the gradual activity in antioxidant enzymes, then in biochemical increase (chlorophyll and phenol) in 3 times or systemic acquired resistance until the last time of sample taken (Agrios 2005).

Phenol content

Data illustrated in Fig. 2 show that the total phenol contents increased gradually up to 15 days after spraying with the tested botanical extracts than in the non-treated control. Lantana extract was the most effective all over the period of study (1, 3, and 15 days) in increasing the total phenolic contents of wheat leaves infected with *P. triticina* to 13.82, 25.81, and 62.76 mg/g fresh weight at 1, 3, and 15 days after spraying, respectively. However, these total phenolic contents in the non-treated control were only 5.33, 5.46, and 8.76 mg/g, respectively (Fig. 2). The henna extract ranked second to the lantana extract (9.77, 19.26, and 53.92 mg/g), followed by chinaberry (9.48, 18.78, 51.41 mg/g) and the fungicide "Fungshow" (7.63, 11.95, 33.60 mg/g).

Plants have the ability to synthesize aromatic secondary metabolites, like phenols, phenolic acids, quinones,

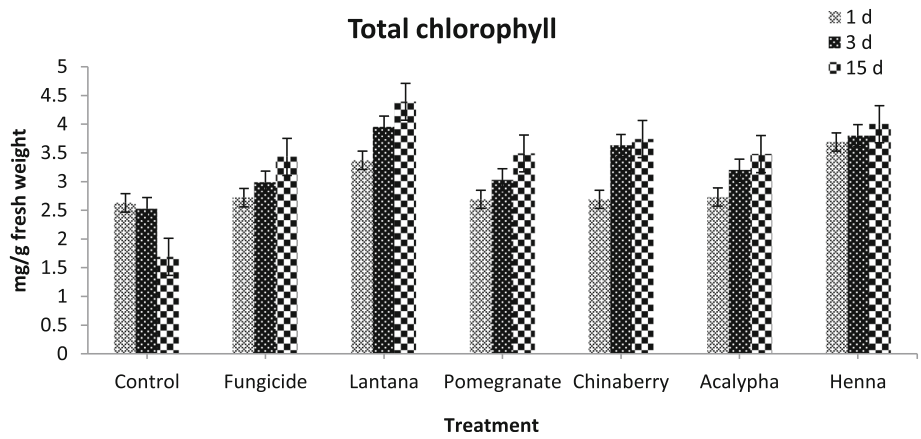

Fig. 1 Effect of some plant extracts on the total chlorophyll (a + b) contents of wheat leaves cv. "Gemmeiza-7" infected with *Puccinia triticina*, as compared to the fungicide Fungshow (diniconazole)

flavones, flavonoids, flavonols, tannins, and coumarins (Cowan 1999). The components with phenolic structures, like carvacrol, eugenol, and thymol, are highly active against pathogens. Obtained findings are in agreement with Karavaev et al. (2002) who reported that the aqueous extracts from bird cherry tree *Padus avium*, aspen *Populus tremula*, and celandine *Chelidonium majus* effectively suppressed the *P. triticina* and antifungal activity was attributed to the high content of phenolic compounds in the leaves of these plants.

Oxidative enzyme activity

Oxidative enzymes were increased on the 1st and 3rd days after spraying at all treatments, then declined as well as in the non-treated control (Figs. 3 and 4). The botanical extracts increased the activity of peroxidase (POX) and polyphenoloxidase (PPO). Lantana extract treatment was the most effective in enhancing the activity of POX, followed by henna and chinaberry extracts all over the period of study (1, 3, and 15 days after exposure). On the other hand, acalypha and pomegranate extracts had the lowest efficacy (Fig. 3). Data in Fig. 4 illustrates that spraying *P. triticina*-infected wheat plants with the tested plant extracts increased PPO activity. Lantana extract was the most effective, followed by each of henna and chinaberry extracts, compared to the non-treated and infected control. The lowest effective treatment in this respect was by acalypha extract, followed by pomegranate extract and fungicide.

The underlying mechanisms of disease suppression by plant extracts are not clearly understood, but the involvement of induced resistance is considered (Fokkema 1993). In the present work, significant increases in POX and PPO recorded at the plant extract treatments were supported by Kumar et al. (2017). Karavaev et al. (2002) recorded a high POX activity in the wheat leaves treated

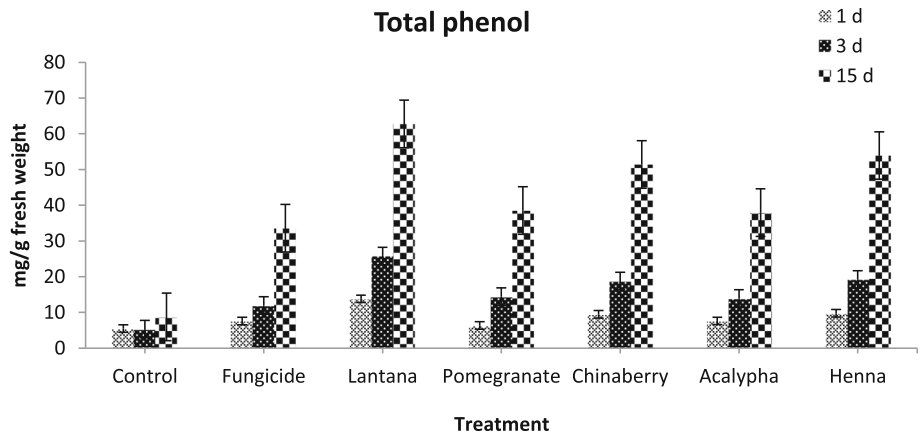

Fig. 2 Effect of some plant extracts on the total phenolic contents of wheat leaves cv. "Gemmeiza-7" infected with *Puccinia triticina*, as compared to the fungicide Fungshow (diniconazole)

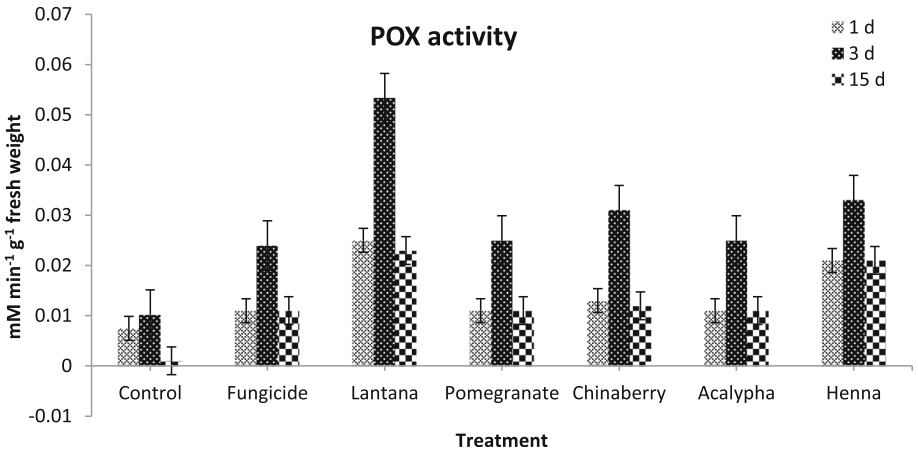

Fig. 3 Effect of some plant extracts on the activity of peroxidase (POX) enzyme in wheat leaves cv. "Gemmeiza-7" infected with *Puccinia triticina*, as compared to the fungicide Fungshow (diniconazole)

with the aqueous extracts from bird cherry tree, *Padus avium*, aspen *Populus tremula*, and celandine *Chelidonium majus*, to suppress the *P. triticina*. Kumar et al. (2017) recorded the maximum PPO activity in *L. camara*-treated potato leaves. Also, willow aqueous extracts reduced the disease incidence of Fusarium wilt in tomato seedlings by inducing the activities of antioxidant defensive enzymes and decreasing the level of lipid peroxidation after 3 and 7 days of infection (Farag et al. 2011).

The phenomenon of inducing resistance in plants by abiotic compounds includes plant extracts that are more environmentally approached for crop protection against infection with many diseases (Morsy et al. 2011). Swamy et al. (2015) mentioned that extract of *L. camara* contained 32 bioactive components as revealed by GC-MS study. Satya Prasad et al. (2015) reported that the extract of *A. indica* has high antioxidant activity on phytochemicals, low LC_{50} value, and the presence of carbohydrates, amino acids, proteins, tannins, flavonoids, anthocyanins and Î²-cyanins, quinones, glycosides, and phenols in aqueous, methanol, and chloroform extracts. Awe et al. (2013) analyzed the leaf extract of *A. wilkesiana* and revealed a high presence of tannins and glycoside, a moderate presence of saponin, flavonoids, phylobatanins, and glycosides and slight presence of alkaloids and cardiac glycosides. These groups of compounds serve as plant defense mechanisms against leaf rust of wheat. The plant extracts are nonpolluting, cost-effective, and non-hazardous and can be prepared with available materials in the field.

Conclusion

It may be concluded from the present findings that prior-infection treatments with the plant extracts (henna, lantana, chinaberry, acalypha, and pomegranate)

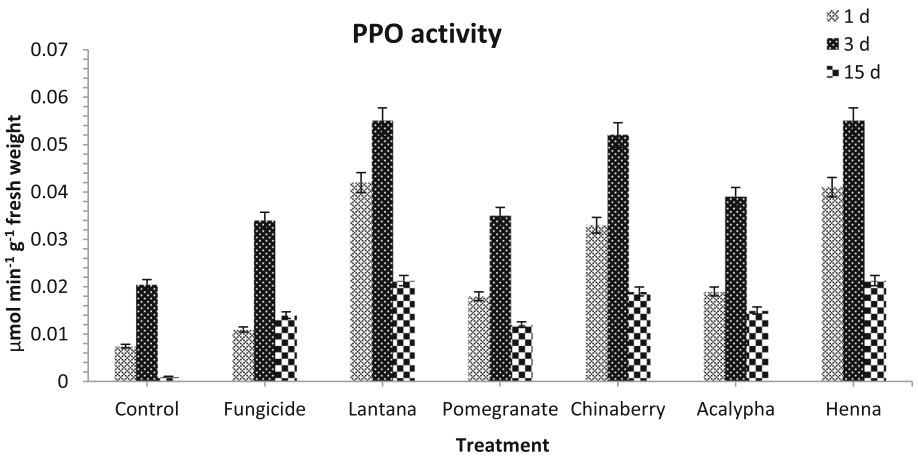

Fig. 4 Effect of some plant extracts on the activity of polyphenoloxidase (PPO) enzyme in wheat leaves cv. "Gemmeiza-7" infected with *Puccinia triticina*, as compared to the fungicide Fungshow (diniconazole)

gave an evidence to play an important role as inducers to resist the leaf rust of wheat caused by *P. triticina*. The tested plant extracts decreased disease severity through biochemical changes in wheat leaves resulting in increased chlorophyll, phenol contents, and oxidative enzymes activities of POX and PPO that are responsible for protecting wheat from leaf rust infection. Prior application of plant inducers to challenge inoculation sensitized the plants to produce an elevated level of defense-related enzymes like peroxidase and polyphenoloxidase.

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Availability of data and materials
All data generated or analysed during this study are included in this published article.

Authors' contributions
All authors conceived the presented idea, developed the theory, and performed the analysis. Elkhwaga AA carried out the experiments under supervision by co-authors. Draz IS verified the analytical methods, investigated the findings, and wrote the manuscript with input from co-authors. All authors discussed the results and contributed to the final manuscript. All authors read and approved the final manuscript

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Ethics approval and consent to participate
Not applicable.

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Competing interests
The authors declare that they have no competing interests.

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