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ORIGINAL PAPER

Biological Induction of Metabolic Defense Mechanisms Against Wheat Yellow Rust Using Bacterial and Organic Biocontrol Agents

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ABSTRACT

Six biocontrol agents, including bacterial antagonists; *Pseudomonas fluorescens* (strain OR485143), *Arthrospira platensis* and *Nostoc muscorum*, as well as organic compounds; fulvic acid, cinnamic acid and 8-Quinolinol (8-hydroxyquinoline) were tested as biological inducers against yellow rust of wheat (cv. Gemmeiza-11). In two field trials, the six biological treatments each proved to be an effective antifungal agent for inducing an adequate level of resistance against wheat yellow rust. The pre-infection application resulted in a lower host response to moderate susceptibility, as well as a significant decrease in average coefficient of infection on adult plants, and subsequently increased grain yield components. The most effective treatment was 8-Quinolinol, which offered a good level of disease protection (reached 92% efficacy), followed by spraying of cinnamic acid, *A. platensis* and *N. muscorum* (89.33% efficacy each), in comparison with the fungicide propiconazole 25% (94.66% efficacy). Meanwhile, *P. fluorescens* and fulvic acid offered relatively low protection of 84% each. Metabolic assay of wheat leaves treated with the six biological inducers resulted in a significant accumulation of total phenolic content and an increase in the antioxidant enzymatic activity of POX and PPO enzymes. This study provides profitable results on the capability use of the tested biological agents to induce an adequate level of bioprotective effect against wheat yellow rust, as a safe and eco-friendly alternative to the synthetic fungicide application.

Keywords : Wheat , Yellow rust , Induced resistance , Biocontrol agents , Metabolism

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INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops, providing a staple food source for roughly one-third of the world's population. Yellow rust (stripe rust), caused by *Puccinia striiformis* Westend. f. sp.*tritici* Eriks. & E. Henn. (*Pst*), is a major threat to wheat production (**Chen, 2020**). This disease occurs annually in most wheat-growing areas with cool and moist weather (**Ali et al., 2017**). Yellow rust affects more than 88% of the world's wheat production, resulting in global losses of over 5 million tons of wheat grains, with an estimated market value of $USD 1 billion per year (**Beddow et al., 2015**). In Egypt, at least five years ago, new evolution and sudden emergence of virulent races of *P. striiformis* f. sp. *tritici* were recorded, causing severe epiphytotic on multiple cultivated wheat cultivars (**Esmail et al., 2021). G**rain yield loss due to yellow rust artificial inoculation has reached 69.33% in highly susceptible wheat cultivars in Egypt (**Shahin et al., 2020**). Breeding wheat cultivars of a sustainable host-genetic resistance faces more challenges due to uncontrollable factors posed by pathogen virulence evolution, and ambient climatic changes (**Ellis *et al*., 2014**; **Miedaner and Juroszek, 2021)**. Also, the wide use of chemical fungicides has adverse environmental side effects, as well as the resistance build-up and/or the adaptive tolerance of the target pathogen against a wide application of these

fungicides (**Brent and Holloman, 2007**). Hence, there is an urgent need to search for alternative solutions, that can be effectively used to successfully control these dangerous diseases. Biocontrol methods that employ diverse antagonistic microorganisms and natural antagonists to combat plant diseases have emerged as a viable alternative to the use of synthetic fungicides (**El-Ghaouth, 1997 and El-Gremi *et al*., 2017)**.

Keeping in view the major importance of the yellow rust disease, recent investigations were undertaken to appraise the impact of endophytic bacteria; *Pseudomonas fluorescens* and *Bacillus amyloliquefaciens* as biocontrol agents against yellow rust of wheat (**Buttar *et al*., 2020**). Biological treatments for successfully control soil-borne and foliar diseases have been previously achieved by several researchers using fungal, bacterial and yeast biocontrol agents (**Eldoksch *et al*., 2001; Hussein *et al*., 2017**; **El-Gremi *et al*., 2017 and Omara *et al*., 2020**). Moreover, *Bacillus* spp. and *P. fluorescens*, as the biocontrol agents were previously reported to suppress certain pathogenic fungi, causing both soil-borne and foliar diseases, and proved to possess antifungal activity against such plant pathogens (**Yu *et al*., 2011; Muis *et al*., 2017 and El-Kazzaz *et al*., 2020**). Induction of resistance using biological inducers is a promising approach to successfully protect crops from severe infection of some foliar diseases. Biocontrol agents can produce certain antibiotics against the parasitism of particular pathogenic fungi, and induce systemic resistance in their host plants (**Pal and Gardener, 2006; Velivelli *et al*., 2014 and El-Kazzaz *et al*., 2020**).

On the other hand, a class of Prokaryotic microorganisms known as cyanobacteria, or blue-green algae, are widely found in both terrestrial and aquatic habitats. They generate a vast array of bioactive chemicals, the majority of which are utilized in the nutraceutical and pharmaceutical sectors, human and animal nutrition, cosmetics, and biofuel production. Studies on the utilization of cyanobacteria in agriculture have recently highlighted their potential as a natural supply of bioactive substances, such phycobiliproteins, for controlling plant pathogens and as inducers of systemic resistance in plants (**Righini *et al*., 2020 and Righini *et al*., 2022**). *Arthrospira* (*Spirulina*) *platensis* is one of the photoautotrophic, planktonic, filamentous green-blue algae (cyanobacteria) , that have become of major medical interest (**Wollina *et al*., 2018**). *A. platensis* extract proved to be contained phenolics, that are toxic to the causal fungi, and causes antifungal activity (**Seghiri *et al*., 2019; Bancalari *et al*., 2020 and Attia *et al*., 2023**).

Nostocales have been extensively studied since the early 2000s. Several cyanobacterial extracts from *Anabaena* spp., *Microcystis aeruginosa, Fischerella* sp., *Nostoc* spp., *Scytonema* spp., *Lyngbya lutea*, *Synechococcus elongates, Oscillatoria* spp., *Phormidium tenue*, *Trichodesmium hildebrantii*, and *Synechocystis* sp., each has an inhibition antifungal effect for mycelial growth of *Aspergillus* spp. in agar disk diffusion assay (**El-Sheekh *et al*., 2006; Pawar *et al*., 2008 and Shishido *et al*., 2015**). Furthermore, *N. muscorum* is known to contain a number of significant enzymes, including peroxidase, hydrogenase, and alkaline phosphatase, which are empirically employed in the biotransformation of hydrocortisone as an exogenous substrate (**Asakawa and Noma, 2010**).

It is noteworthy mentioned in most of the previous studies that natural organic materials usually refer to the natural products that are characterized as chemical compounds, produced by certain living organisms, and they are generally found in nature (**Bhat *et al*., 2005**). Out of these compounds, cinnamic acid is an organic acid occurring naturally in plants, that has been proven to possess low toxicity, along with a broad spectrum of crucial biological activities. However, cinnamic acid also showed a potential efficacy to be a natural alternative to commercial or synthetic fungicides. This natural compound was utilized to develop a new bio-fungicide for the effective control of Sclerotinia stem rot. The biological characteristics of the natural product; cinnamic acid, contribute to well understanding of the action mechanism of this organic acid and its antifungal activity against *S. sclerotiorum* (**Wang *et al*., 2019**). Meanwhile, the superior biocontrol agent; 8-Quinolinol (8-hydroxyquinoline) is a simple alkaloid that is known for many biological activities.

Application of 8-quinolinol and its derivatives is actually based on their antibacterial (**Albert *et al*., 1953 and Kim *et al*., 2006**), antifungal (**Musiol *et al*., 2010**), antiparasitic (**Kell, 2009**), and insecticidal (**Lee *et al*., 2010**) activities. Likewise, fulvic acid has been previously recorded to have an appositive effect against **some dangerous** plant pathogens (**El-Sawy *et al*., 2016**). This natural product was previously used as a fungicide alternative against the most important rust disease; yellow rust in wheat (**El-Sawy *et al*., 2016**). Also, **Kamel *et al*. (2014)**, and his coworkers, used fulvic acid in their study, as an effective tool for controlling powdery and downy mildews in cucumber plants.

Hence, this study has been primarily aimed to investigate the role of the application of six biological agents, bacterial antagonists and organic materials, for enhancement inducing host-genetic resistance against wheat yellow rust. The second aim was to elucidate the potential impact of the application of these biological agents for increasing wheat grain yield. An ultimate goal was to precisely explore the role of the tested biological agents in inducing some important metabolic defense-related mechanisms against such dangerous disease in wheat.

MATERIALS AND METHODS

This investigation was carried out in two field trials at El-Gemmeiza Agricultural Research Station, Agricultural Research Center (ARC), Egypt, during the two growing seasons; 2021/2022 and 2022/2023. Six biological control agents, including three antagonistic bacteria; *Pseudomonas fluorescens*, *Arthrospira platensis* (*Spirulina platensis*), and *Nostoc muscorum*, as well as three organic compounds; 8-Quinolinol, fulvic acid, and cinnamic acid, were screened as inducers for defense related-mechanisms in wheat plants (cv. Gemmeiza-11) against yellow rust caused by *P. striiformis* f. sp. *tritici*.

Preparation of bacterial antagonists

A representative bacterial isolate of *P. fluorescens*, which used in current study, was kindly obtained from the Integrated Control Research Department, Plant Pathology Research Institute, ARC, Giza, Egypt. Molecular sequencing of bacterial isolate of *P. fluorescens* was performed using the 16S rDNA primer sequence (PA-GS-fwd: GAC GGG TGA GTA ATG CCT A, PA-GS-rev: CAC TGG TGT TCC TTC CTA TA), according to the method described by **Spilker et al. (2004)**. The sequencing process was made by comparing with similar sequences in the NCBI database according to BLAST search (https: //blast. ncbi. nlm. nih.gov /Blast.cgi) . Sequencing of the tested bacterial isolate *P. fluorescens* based on the 16S rDNA in the NCBI database showed identity with *P. fluorescens* strain OR485143.

The bacterial isolate of *P. fluorescens* strain OR485143 was cultured on King's medium and maintained at 28±2ºC for 5 days (**King *et al*., 1954**). A bacterial medium was prepared using different carbon and nitrogen sources, as well as salts that promote bacterial growth and the consequent FP production (**Georgia and Poe 1931, 1932**). The basic ingredients used to prepare the medium were agar (PGS), Bacto peptone, gelatine, dipotassium hydrogen phosphate, sucrose, and magnesium sulfate (**Lamichhane and Varvaro, 2013**).

Cyanobacteria isolates of *A. platensis* and *N. muscorum* that tested in the current study, were kindly obtained from the Microbiology Department, Soil, Water and Environmental Research Institute, ARC, Egypt. Isolates of *A. platensis* and *N. muscorum* were maintained in Zarrouk's liquid medium (**Zarrouk, 1966**) and BG-11 liquid medium (**Rippka *et al*., 1979**), respectively. Cultures were separately maintained in 1 L flasks with 300 ml culture medium composed of sterilized tap water. Each flask was inoculated with 3 ml of cyanobacteria and incubated at 29 ± 2°C under a 12 h light/12 h dark cycle with a light intensity of 156 mmol of photons s^{-1} m^{-2} and constant aeration of 4.95 ± 0.03 ml s^{-1}. Three to four times a day, cultures were manually shaken. Following a 20-day period, the biomass inside the culture medium was extracted using filter paper (Whatman No. 1), with the filtrate output being used as an applied therapy (**Pandey et al., 2010 and Loaiza *et al*., 2016**).

The tested bacterial isolates were separately suspended in sterile distilled water and applied at 10^9 CFU ml^{-1} adjusted using a spectrophotometer at 600 nm.

Preparation of organic biocontrol agents

Fulvic acid (Technogen Chemical Co. Egypt), was prepared according to the methods described by **Kononova (1966)**. Ten grams of dried powder of fulvic acid were extracted with 100 ml distilled water for 24 h at room temperature. The obtained extracts were collected, filtered and concentrated at 40ºC. Subsequently, a stock solution of fulvic acid was 100,000 ppm. The rate of fulvic acid treatment was 10 ml L^{-1}.

Cinnamic acid (96%) and 8-Quinolinol (8-hydroxyquinoline) were obtained from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). The dried powder of each compound was dissolved in sterile distilled water at 0.25 g L^{-1}.

Evaluation of biological agents against wheat yellow rust

Field trails were conducted in the 2021/22 and 202/23 growing seasons at the Experimental Farm of El-Gemmeiza Agricultural Research Station, ARC, Egypt. Susceptible wheat cultivar Gemmeiza-11 grains were sown in random 3-by-2-meter plots, at a rate of 40 g/plot. Three replications were included in the randomized complete block design (RCBD) used for the trials. The biological treatments were given to wheat plants using a hand sprayer at the 7-8th growth stage (**Large, 1954**), until the plants were wet. A comparable control was a synthetic fungicide (propiconazole 25%) at 0.25 mL L^{-1}. In addition, the unsprayed plants acted as a control (untreated) treatment. The application was done one day before the inoculation with *P. striiformis* f. sp. *tritici* urediniospores. The inoculation procedure was performed using a urediniospore powder mixture of *Pst* isolates, according to **Tervet and Cassel (1951)**. Only distilled water was sprayed on the untreated (control) plants.

Disease assessment was conducted using the five infection types; immune (0), resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible (S), as described by **Roelfs *et al*. (1992)**. Disease severity (%) was quantified as the percentage of rust pustules covering of leaves as indicated by **Peterson et al. (1948)**. The average coefficient of infection (ACI) values, were determined by multiplying disease severity (%) by the constant values of infection types (R = 0.2, MR = 0.4, MR-MS = 0.6, MS = 0.8, and S = 1.0), according to **Saari and Wilcoxson (1974)**.

The efficacy (%) of each treatment under consideration was calculated using **Rewal and Jhooty's (1985)** equation:

$$\textbf{Efficacy (\%)} = (C - T/C) \times 100$$

Where:

C = Average coefficient of infection (ACI) in the untreated (control).

T = Average coefficient of infection (ACI) in the treatment.

Impact of biological treatments on wheat grain yield components

The impact of the studied biological treatments on wheat grain yield components was assessed at harvest in terms of spike weight (g), 1000-kernel weight (g), and volume weight (g L^{-1}).

Metabolic defense mechanisms

Metabolic analysis of yellow rust-infected leaves of wheat plants (cv. Gemmeiza-11), treated with the six biological agents of the study, was carried out in the Integrated Control Research Department, Plant Pathology Research Institute, ARC, Giza, Egypt. Fresh leaves of adult plants representing each treatment and the untreated (control) were sampled on the 1st, 3rd, and 15th day post inoculation (dpi), to ascertain the impact of each treatment on certain biochemical components, such as total phenolic content and antioxidant enzyme activity,

Estimation of phenolic content

The method outlined by Malik and Singh (1980) was used to assess the total phenolic content. 0.5 g of fresh leaves were crushed with 10 ml of 80% ethanol and kept for 72 hours at 4°C in a dark bottle. The extracts were mixed and filtered using a Unico UV-2100 Spectrophotometer. Folin-Ciocâlteu reagent was used to measure the total amount of phenol, and a 650 nm wavelength was used to measure the absorbance. The total phenolic content was given as mg g^{-1} fresh weight (FW).

Estimation of oxidative enzyme activity

Direct measurement of peroxidase (POX) activity was conducted using a standard protocol that was suggested by **Hammerschmidt et al. (1982)**. Polyphenoloxidase (PPO) activity, however, was measured using the technique outlined by **Malik and Singh (1980)**. It involved homogenizing 0.5 g of leaf material at 0–4°C in 3 ml of 50 Mm TRIS buffer (PH 7.8) that contained 7.5% polyvinylpyrrolidone and 1 mM EDTA-Na2. After centrifuging the homogenates for 20 minutes at 4°C at 12,000 rpm, the total soluble enzyme activities in the supernatant were measured at 25°C using spectrophotometry (model UV-160A, Shimadzu, Japan) with three iterations. For three

minutes, 30-s intervals were used to record changes in absorbance at 470 nm for POX activity and 495 nm for PPO activity. The expression for enzyme activity was the increase in absorbance as min^{-1} g^{-1} FW.

Statistical analysis

Data were subjected to the combined analysis of variance (ANOVA), using the Statistical Analysis System package (SAS software v.9.2). The least significant difference (LSD) test at *P* 0.05 (**Steel and Torrie, 1980**) and Duncan multiple range test (**Duncan 1954**) were used to compare means.

RESULTS

To investigate, more precisely, the role of the six biocontrol agents under study, to enhance yellow rust resistance in wheat plants of the susceptible cv. Gemmeiza-11, the evaluated biocontrol agents were applied as a pre-infection foliar spray for the tested plants, under field conditions at El-Gemmeiza Agric. Res. Stn., during the two growing seasons; 2021/22 and 2022/23. The critical effect of the six biocontrol agents under evaluation was empirically determined or measured by their effects in decreasing or minimizing the incidence of the two disease parameters *i.e*. adult plant response and average coefficient of infection (ACI), compared to the application of the synthetic fungicide and an untreated (control) treatment. However, all of the obtained data were subjected to the combined analysis of variance, as outlined in Table (1).

The combined analysis of variance (Table 1) recorded a highly significant difference between treatments (T) as well as between seasons (S), while the difference between their interaction (T × S) was found to be non-significative (ns). Due to the insignificant difference in the interaction between treatment and season (T × S), L.S.D. value was used to compare the mean of each treatment.

Efficacy of the tested biocontrol agents against wheat yellow rust, under field conditions

Data in Table (2) showed that pre-infection spraying of wheat plants (cv. Gemmeiza-11) with any of the six biocontrol agents, under evaluation, had an effective role against yellow rust infection, at adult plant stage, during the two growing seasons; 2021/22 and 2022/23. The relatively low adult plant response of wheat plants was observed after an application of 8-Quinolinol with moderately susceptible (MS) response ranged from 5 MS to 10 MS, followed by cinnamic acid, *A. platensis* and *N. muscorum*, (10 MS for each), during both seasons. Also, moderately susceptible response was detected with each of *P. fluorescens* and fulvic acid, but it was ranged from 10 MS to 20 MS. Similarly, low adult plant response was recorded with the application of the synthetic fungicide, propiconazole 25% (Tr S to 5 S), but it was in susceptible (S) response.

Table (1): Combined analysis of variance (ANOVA) of the combined data for treatments (T), seasons (S) and their interactions (T × S) for disease severity (%) and average coefficient of infection (ACI) of yellow rust in wheat (cv. Gemmeiza-11) treated with the tested biological agents.

Source of variation	DF	Disease severity		ACI	
		MS	F	MS	F
Treatments (T)	7	3217.17	68.63 **	3534.85	153.68 **
Seasons (S)	1	507	10.81 **	330.75	14.38 **
Interaction (T × S)	7	24.85	0.53 ns	13.60	0.59 ns
Error	32	46.87		23	

*significant ($p < 0.05$), ** highly significant ($p < 0.01$), (ns) non-significant ($p >= 0.05$)

In contrast, untreated (control) wheat plants exhibited a highly susceptible response, rating 80 S and 70 S, during both seasons of the study, respectively. On the other hand, the average coefficient of infection (ACI) was significantly reduced by the pre-treatment with the tested biocontrol agents, as compared to untreated (control) treatment. The lowest ACI values of 8 and 4were recorded with 8-Quinolinol foliar spraying, during the two seasons of the study, respectively, followed by *A. platensis*, *N. muscorum*

and cinnamic acid with ACI values of 8 for each. While, the application of *P. fluorescens* and fulvic acid resulted in the ACI values of 16 and 8 for each, during the first and second seasons, respectively. The average coefficient of infection (ACI) as the calculated means of two seasons was reduced to 6 by 8-Quinolinol and to 8 by each of *A. platensis*, *N. muscorum* and cinnamic acid, which was insignificantly different

Table (2): Effect of the six biological control agents on yellow rust in wheat plants (cv. Gemmeiza-11), under field conditions during the two growing seasons (2021/22 and 2022/23), as compared to the fungicide (propiconazole 25%) application, and untreated (control) treatment.

Treatment	Adult plant response			ACI*			Efficacy (%)**
	1st season	2nd season	Mean	1st season	2nd season	Mean	
P. fluorescens	20 MS	10 MS	15 (MS)	16	8	12	84
A. platensis	10 MS	10 MS	10 (MS)	8	8	8	89.33
N. muscorum	10 MS	10 MS	10 (MS)	8	8	8	89.33
8-Quinolinol	10 MS	5 MS	7.5 (MS)	8	4	6	92
Cinnamic acid	10 MS	10 MS	10 (MS)	8	8	8	89.33
Fulvic acid	20 MS	10 MS	15 (MS)	16	8	12	84
Fungicide	5 S	Tr S	4 (S)	5	3	4	94.66
Control (untreated)	80 S	70 S	75 (S)	80	70	75	–
LSD at 0.05:							
Treatments (T)			8.04			5.63	
Seasons (S)			4.02			2.81	
Interaction (T ×S)			ns			ns	

*ACI = average coefficient of infection. S = susceptible, MS = moderately susceptible, Tr (traces) = 3.
** Efficacy (%) was estimated for each treatment, according to Rewal and Jhooty (1985).

from the fungicide treatment (ACI=4) . *P. fluorescens* and fulvic acid each recorded an ACI value of 12. However, ACI value for the untreated (control) treatment reached up to 75. All the tested biocontrol agents were very effective in reducing adult plant response and the ACI values on the infected treated wheat plants, with efficacy ranging from 84 to 92%. 8-Quinolinol was the most effective treatment (92%), followed by *A. platensis*, *N. muscorum* and cinnamic acid (89.33% for each), which were comparable to the fungicide propiconazole-25% (94.66% efficacy). *P. fluorescens* and fulvic acid each demonstrated 84% efficacy.

Impact of biological inducer treatments on grain yield components

Analysis of variance for the combined data obtained during both seasons of the study (Table 3) recorded highly significant difference between treatments (T), seasons (S), and their interactions (T × S) for spike weight and 1000-kernel weight.

Meanwhile, difference in volume weight was highly significant between treatments (T) and only

Table (3): Combined analysis of variance (ANOVA) of treatments (T), seasons (S) and their interactions (T × S) for grain yield components in wheat cv; Gemmeiza-11 treated with the tested biological agents.

Source of variation	DF	Spike weight		1000-kernel weight		Volume weight	
		MS	F	MS	F	MS	F
Treatments (T)	7	1.87	1167.50 **	335.68	6420.67 **	5203.27	1595.73 **
Seasons (S)	1	0.22	142.10 **	2.62	50.16 **	16.45	5.04 *
Interaction (T × S)	7	0.03	21.12 **	0.17	3.29 **	0.83	0.25 ns
Error	32	0.001		0.05		3.26	

* significant ($p < 0.05$), ** highly significant ($p < 0.01$), ns non-significant ($p >= 0.05$)

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significant between seasons (S), but non-significant (ns) between their interaction (T × S). Considerable and/or highly significant differences were obtained between the evaluated biological agent treatments and untreated (control) treatment. Data in Table (4) show that all biological treatments, under evaluation in this study, significantly increased the grain yield components; as weight of spike (g), 1000-kernel weight (g), and volume weight (g L^{-1}), in comparison with the untreated (control) treatment, during the two growing seasons *i.e.* 2021/22 and 2022/23. Among the biological treatments; 8-Quinolinol application resulted in the highest weight of the studied grain yield components, in the calculated means of both seasons, *i.e*. a spike weight of 4.74 g, 1000-kernel weight of 64.59 g, and a volume weight of 734.51 g. Similarly, cinnamic acid ranked second (4.62, 63.60, 731.03 g), followed by *A. platensis* (4.50, 60.39, 720.94 g) and *N. muscorum* (4.43, 59.33, 720.55 g), for the tested three grain yield components, respectively. The aforementioned treatments were comparable to the synthetic fungicide; propiconazole 25%, which

Table (4): Effect of the six biological inducers on grain yield components of wheat (cv. Gemmeiza-11) inoculated with yellow rust, under field conditions during the two growing seasons (2021/22 and 2022/23), as compared to the fungicide (propiconazole 25%) and untreated (control) treatment.

Treatment	Spike weight (g)		Mean	1000-kernel weight (g)		Mean	Volume weight (g L^{-1})		Mean
	1st season	2nd season		1st season	2nd season		1st season	2nd season	
P. fluorescens	3.98	4.04	4.01	56.06	56.46	56.26	701.15	701.17	701.16
A. platensis	4.45	4.56	4.50	60.17	60.61	60.39	720.21	721.66	720.94
N. muscorum	4.42	4.45	4.43	59.14	59.53	59.33	720.88	720.22	720.55
8-Quinolinol	4.54	4.94	4.74	64.35	64.83	64.59	734.28	734.74	734.51
Cinnamic acid	4.51	4.74	4.62	63.57	63.63	63.60	729.90	732.16	731.03
Fulvic acid	4.20	4.32	4.26	58.51	59.58	59.04	710.79	713.00	711.89
Fungicide	4.44	4.53	4.49	59.54	60.40	60.39	711.95	713.26	712.60
Control (untreated)	2.98	3.03	3.01	40.62	40.66	40.64	640.83	642.31	641.57
LSD at 0.05									
Treatments (T)			0.047			0.268			2.12
Seasons (S)			0.023			0.134			1.06
Interaction (T × S)			0.066			0.379			Ns

1st season = 2021/22 growing season, 2nd season = 2022/23 growing season

recorded a spike weight of 4.49 g, 1000-kernel weight of 60.39 g, and a volume weight of 712.20 g. While, fulvic acid and *P. fluorescens* each recorded a relatively low increase in the three-grain yield components, during both growing seasons. Contrarily, the lowest weight of each grain yield component under study, was observed with untreated (control) treatment.

Metabolic defense mechanisms

Total phenolic content

Data in Fig. (1) indicate that the total phenolic content was significantly increased gradually in yellow rust-infected wheat leaves (cv. Gemmeiza-11), up to 15 dpi after treatment with the six biological inducers, as compared to the fungicide (propiconazole 25%) and untreated (control) treatment. The highest concentration of total phenol was recorded with 8-Quinolinol treatment all over the periods of 1, 3 and 15

dpi, being 22.86, 34.03 and 65.72 mg g^{-1} fresh weight (FW), respectively. Likewise, cinnamic acid ranked the second biocontrol agent, recording a total phenol of 18, 32.68 and 55.08 mg g^{-1} FW, followed by fulvic acid (11.95, 32.06, 55.08 mg g^{-1} FW), *A. platensis* (11.95, 32.06 and 54.08 mg g^{-1}), and *P. fluorescens* (11.3, 30.21, 52.04 mg g^{-1} FW). Except for *N. muscorum* (9.6, 18.01, 45.06 mg g^{-1} FW), the aforementioned biocontrol agents were comparable to the synthetic fungicide treatment, showing a total phenol of 9.8, 18.52, and 47.29 mg g^{-1} FW. In contrast, a noticeable decrease in total phenol was recorded with untreated (control) treatment (5.46, 10.46 and 25.95 mg g^{-1} FW), in the three times of application, respectively.

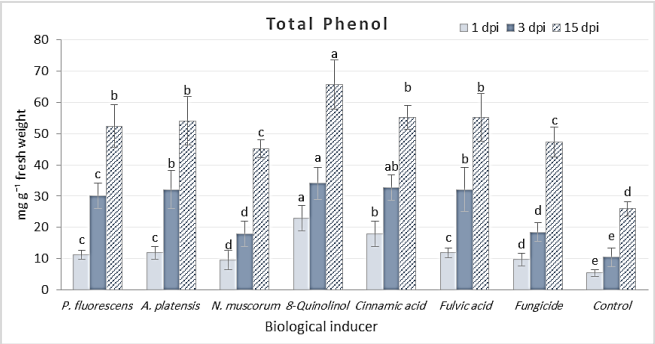

Fig. (1): Total phenolic contents in yellow rust-infected wheat leaves (cv. Gemmeiza-11) treated with the six biocontrol agents (inducers), as compared to the fungicide (propiconazole 25%) application and untreated (control) treatment.

Antioxidant enzyme activities

Data in Figs. (2 and 3) reveal, in general, that there was a significant increase in the enzymatic activities of the two enzymes *i.e*. peroxidase (POX) and polyphenoloxidase (PPO) in yellow rust-infected wheat leaves (cv. Gemmeiza-11), treated with each of the six biological inducers, under evaluation, particularly at 1 and 3 dpi. While, these enzymatic activities have been noticeably declined at 15 dpi, as compared to those found due to the fungicide (propiconazole 25%) treatment and untreated (control) wheat leaves. The highest activity of POX enzyme was observed after the application of 8-Quinolinol treatment, all over the three times of application, 1, 3 and 15 dpi, recording 0.025, 0.045 and 0.025 µmol min^{-1} g^{-1} fresh weight (FW), respectively (Fig. 2). Also, cinnamic acid ranked the second superior biocontrol agent, recording a POX activity of 0.02, 0.037 and 0.017 µmol min^{-1} g^{-1} FW, followed by *N. muscorum* and fulvic acid with a POX activity of 0.015, 0.036 and 0.012 µmol min^{-1} g^{-1} FW for each (Fig. 2). In contrast, *A. platensis* and *P. fluorescens* recorded relatively low significant activity of POX enzyme. Except for *P. fluorescens*, the aforementioned treatments in comparison with fungicide propiconazole 25% recorded POX activity of 0.015, 0.035 and 0.012 µmol min^{-1} g^{-1} FW (Fig. 2). The lowest activity of POX enzyme was recorded with untreated (control) wheat leaves all over the three times application (0.004, 0.008, 0.005 µmol min^{-1} g^{-1} FW).

The highest activity of PPO enzyme was detected with the 8-Quinolinol application at the three times of application of 0.035, 0.058 and 0.036 µmol min^{-1} g^{-1} FW, compared to the recorded activities of the enzyme in the untreated (control) treatment (0.011, 0.014 and 0.009 µmol min^{-1} g^{-1} FW). Also, *N. muscorum* ranked the second effective biocontrol agent (0.028, 0.048, 0.029 µmol min^{-1} g^{-1} FW), followed by fulvic acid and cinnamic acid (Fig 3). The aforementioned treatments were comparable to the fungicide; propiconazole 25% (0.026, 0.042 and 0.022 µmol min^{-1} g^{-1} FW). Application of each of *A. platensis* and *P. fluorescens* showed a relatively low significant increase in PPO activity (Fig. 3). The lowest enzyme activity of POX was recorded with untreated (control) all over the three times of application (0.011, 0.014, and 0.009 µmol min^{-1} g^{-1} FW).

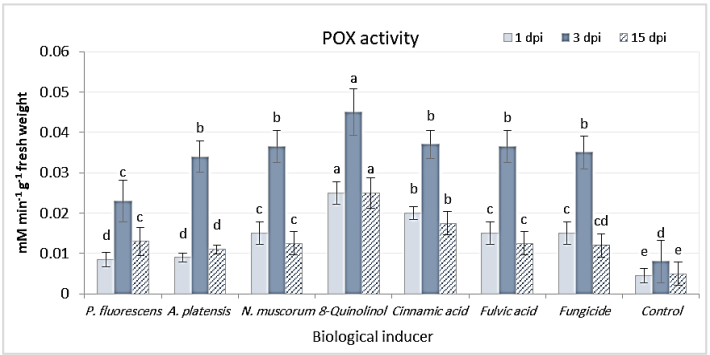

Fig. (2): Peroxidase (POX) enzyme activity in yellow rust-infected wheat leaves (cv. Gemmeiza-11) treated with the six biocontrol agents (inducers), as compared to the fungicide (propiconazole 25%) application and untreated (control) treatment.

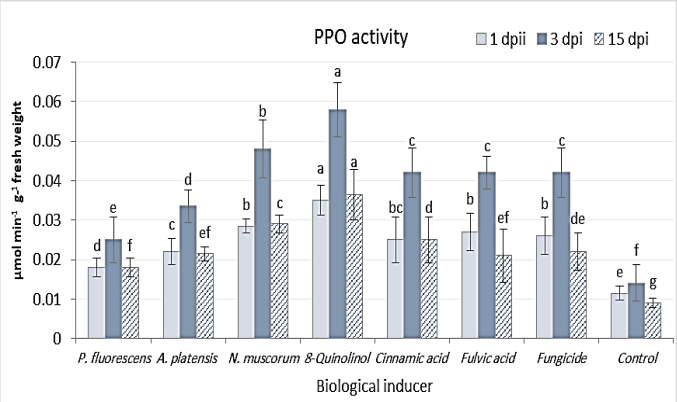

Fig. (3): Polyphenoloxidase (PPO) enzyme activity in yellow rust-infected wheat leaves (cv. Gemmeiza-11) treated with the six biocontrol agents (inducers), as compared to the fungicide (propiconazole 25%) application and untreated (control) treatment.

DISCUSSION

Wheat rust diseases, especially yellow (stripe) rust have been considered to be a serious threat to both quantity and quality of wheat production, food security, and human health globally, necessitating the need to search and discover new antifungal agents with high efficiency. However, eco-friendly biological control means of inducing host plants to resist their particular pathogens is one of the principal sustainable development goals (SDGs) increasingly required. In the current study, six biocontrol agents, including three bacteria, *P. fluorescens* (strain OR485143), *A. platensis*, and *N. muscorum*, as well as three organic compounds; 8-Quinolinol, cinnamic acid, and fulvic acid, were tested to induce host resistance against wheat yellow rust caused by *P. striiformis* f. sp. *tritici*, under field conditions. The tested biological agents were applied to adult wheat plants one day before inoculation. During this study, an application of 8-Quinolinol empirically induced a high level of host resistance against yellow rust disease incidence and/or development in wheat leaves treated with this biocontrol agent along with the treated leaves with the fungicide (propiconazole 25%), compared to untreated (control) treatment. Application of cinnamic acid, *A. platensis*, *N. muscorum*, ranked second as effective biocontrol agents against wheat yellow rust. Meanwhile, application of *P. fluorescens* (strain OR485143) and fulvic acid resulted in relatively low to moderate levels of adult plant resistance against yellow rust in the treated plants of the susceptible wheat cv. Gemmeiza-11, under field conditions. The average coefficient of infection (ACI) as recorded in the wheat plants (cv. Gemmeiza-11) indicated that all biological treatments significantly reduced disease infection, expressed as low ACI values, compared to untreated (control) wheat leaves of the same cv. Gemmeiza-11. The best and/or the superior biocontrol agent was the application of 8-Quinolinol which considerably reduced ACI values to 6, followed by *A. platensis*, *N. muscorum* and cinnamic acid (ACI = 8 each), compared to the untreated (control) treatment (ACI = 75). The aforementioned four biocontrol treatments used were comparable to the fungicide treatment (ACI = 4). These treatments offered or resulted in good levels of rust disease protection, reaching its maximum efficacy to 92% by application of 8-Quinolinol, and 89.33% by each of *A. platensis*, *N. muscorum* and cinnamic acid, which were comparable to the fungicide treatment (94.66%). Meanwhile, application of each of *P. fluorescens* (strain OR485143) and fulvic acid was found to be less effective, which reduced ACI values to 12. Spraying of *P. fluorescens* (strain OR485143) and fulvic acid also provided disease protection of 84% efficacy each. All the tested biological agents under evaluation could elicited different levels of resistance to wheat yellow rust pathogen, and subsequently caused or resulted in an increase for grain yield components, in terms of higher spike weight (g), 1000-kernel weight (g), and volume weight (g L^{-1}), compared to untreated (control) wheat plants. The highest significant increase in grain yield components was recorded with the application of the superior biocontrol agent *i.e*. 8-Quinolinol, followed by cinnamic acid, *A. platensis* and *N. muscorum*, along with the spraying application of fungicide used. While, fulvic acid and *P. fluorescens* (strain OR485143) recorded a relative increase in grain yield components, compared to the untreated (control) wheat plants.

Induced resistance (IR) as reported in the previous studies, has been characterized by an increase and accumulation of some synthesized antimicrobial compounds in the treated leaves of the host plants, that can inhibit the growth and development of the causal pathogen, due to the enhancement or induction of the activity of antioxidant enzymes. Additionally, this causes a biochemical rise in phenol and chlorophyll contents in the treated leaves over three times, which consequently induces systemic resistance against the target pathogen (**Agrios 2005**). Our findings demonstrated the induction of some metabolic defense mechanisms, in terms of a significant increase in the amount of total phenolic content, and enhancement of POX and PPO enzyme activities in yellow rust-infected wheat leaves, treated with the tested biological

agents, as compared to untreated (control) treatment. The maximum and/or considerable increase in total phenol was recorded with the application of 8-Quinolinol treatment, followed by cinnamic acid, fulvic acid, *A. platensis* and *P. fluorescens* (strain OR485143), respectively. Also, the highest increase in POX activity was detected with the application of 8-Quinolinol, followed by cinnamic acid, *N. muscorum* and fulvic acid, respectively. Likewise, the highest enzymatic activity of PPO was observed with the application of 8-Quinolinol, followed by *N. muscorum*, fulvic acid and cinnamic acid, respectively. The above-mentioned treatments were comparable to the application of the synthetic fungicide; propiconazole 25% treatment.

A novel antimicrobial biocontrol agent, 8-Quinolinol (oxine, 8-hydroxyquinoline) was previously characterized in several studies as a simple aromatic alkaloid with allelopathic, antibacterial, antifungal, insecticidal, antiparasitic, and cytotoxic activities (**Kim *et al*., 2006; Kell, 2009; Musiol *et al*., 2010; Lee *et al*., 2010)**. The beneficial effects of 8-Quinolinol and its derivatives in the medication of certain degenerative diseases have long been recognized. Generally, it is assumed that toxicity of 8-Quinolinol to the fungal pathogens depends, to a large extent, on transition metal chelation, which negatively affects the availability of metalloenzymes in the cell or reactive oxygen species generation (ROS), which are formed following reduction of molecular oxygen by autoxidation of the redox active metal central atom of the 8-quinolinol complex (**Chobot *et al*., 2011**).

Although, the search for new antibiotics is imperative due to the emergent resistance of new microorganism strains, the 8-hydroxyquinoline derivatives nitroxoline and clioquinol could use to treat certain microbial infections (**Joaquim *et al*., 2021**). In this situation, going back to previously identified classes, such as 8-hydroxyquinolines, may be an interesting strategy for discovering novel biocontrol agents. The biological activities of the alkaloid 8-hydroxyquinoline are widely recognized, including its antioxidant properties. It is particularly well-known for its ability to form coordination complexes with different transition metals, including iron. A recent study of **Yin *et al*. (2019)**, tested the antifungal activity of a series of 8-hydroxyquinoline derivatives against five phytopathogenic fungi. *In vitro* assays, it was revealed that most of the tested compounds remarkably impacted the five target fungi, *B. cinerea*, *F. graminearum*, *S. sclerotiorum*, *F. oxysporum* and *M. oryzae* and their inhibitory activities were better than that of the positive control azoxystrobin.

Likewise, an organic acid that is frequently utilized as a food additive in the food industry is cinnamic acid. Furthermore, it possesses antibacterial action *in vitro*; nevertheless, its impact in regulating fruit decay remains unclear (**Sova, 2012**). *Botrytis cinerea*-caused gray mold on table grapes was considerably reduced by applying cinnamic acid as a biocontrol agent (**Zhang et al., 2015**). Cinnamic acid has also been shown to directly prevent mycelial development of *B. cinerea* on potato dextrose agar plates. The aforementioned study investigated the processes by which cinnamic acid hindered fungal development because it can compromise the integrity of the plasma membrane and raise intracellular reactive oxygen level, which in turn caused the growth rate to decrease. The enzymatic activities of POX and PPO in the treated plants, which are closely linked to the host resistance, were shown to be greatly stimulated by cinnamic acid treatment (**Sova, 2012; Zhang et al., 2015**). Cinnamic acid significantly suppressed the hyphal growth of *Fusarium oxysporum* f. sp. *niveum*, the fungus that causes watermelon wilt (**Wu et al., 2008**). According to their research, cinnamic acid reduced the hyphal biomass of the causative pathogen in liquid culture by 63.3%. Conversely, there was a total inhibition of colony diameter, conidial germination, and conidial production. On the other hand, there was a significant increase in mycotoxin synthesis and phytopathogenic enzyme activity. Since there was an increase in the yield of mycotoxin, pectinase, proteinase, cellulase, and amylase activities. Cinnamic acid significantly increased the hydrolytic enzyme activities and mycotoxin generation by *F. oxysporum* f. sp. *niveum*, but it reduced the spores' development and germination.

Similar results have been previously obtained concerning the other important biocontrol agent *i.e*., fulvic acid, whereas it has been recorded to have an appositive effect against some of the major important

plant pathogens (**Kamel *et al*., 2014 and El-Sawy *et al*., 2016**). Using some natural products as fungicide alternatives against stripe rust, **El-Sawy et al. (2016)**, and his coworkers, revealed that an organic compound; fulvic acid has precisely proved to have an appositive effect against wheat yellow rust pathogen, *P. striiformis* f. sp. *tritici.* They added that the application of this biocontrol agent precisely enhanced and/or increased yellow rust resistance in the treated wheat plants. Consequently, increased and relatively improved the grain yield of the treated wheat plants compared to the untreated and infected (control) wheat plants. On the other hand, treatment with fulvic acid in rapeseed subsequently increased the chlorophyll content and photosynthesis intensity and simultaneously decreased the permeability of the cell membrane (**Marosz, 2009**).

Antimicrobial-producing bacterial strains, either alone or in combination with fungicide, greatly reduced the stress and/or severity of plant diseases. The important group of bacteria, that play a major role in plant growth promotion, induced systemic resistance, and achieved a successful biological control of diverse plant pathogens. These microorganisms may potentially compete with pathogens for nutrition resources. One of the key mechanisms by which the biocontrol agents are recognized is the use of antibiotics such as pyoluteorin, pyrrolnitrin, phenazine, 2,4, diacetylphloroglucinol (DAPG), amphisin, oomycin A, tropolone, tensin, compound and cyclic lipopeptides (**Gade and Koche, 2022**). The efficacy of bacterial antagonists, as the promising biocontrol agents, for controlling several fungal diseases was often of better effect as alone, and sometimes in combination with particular fungicides. Future research programmes should be aim to promote *P. fluorescens* as a potential bio-fungicide for augmentative biological control of many fungal diseases of agriculture and horticulture. Many strains of *P. fluorescens* are known to enhance plant growth promotion and mainly reduce the severity of various plant diseases (**Ganeshan and Kumar 2005; Gade and Armarkar, 2011)**. A recent study of **El-Kazzaz *et al*. (2020)**, reported that *P. fluorescens* significantly reduced the disease severity (%) of wheat yellow rust, under greenhouse conditions. Also, this important biocontrol agent significantly minimized the average coefficient of infection (ACI), under field conditions. They also found that *P. fluorescens* inhibited the germination of urediniospores of *P. striiformis tritici*, and sharply increased and/or enhanced the activity of peroxidase, polyphenol oxidase enzymes, and the accumulation of total phenol contents. They ultimately found that grain yield components in the tested wheat cultivars were significantly increased due to the application of *P. fluorescens*. *P. fluorescens* strains are the most ideal for using in the field as a disease suppressor and plant growth stimulant. However, they are also generating compounds such as siderophores, antibiotics, and an increase in enzymes, either by inducing systemic resistance in the host plants, or by other means.

A. platensis extract contains phenolic compounds, that are considered to be toxic materials to several plant pathogens, resulting in their antifungal activity (**Seghiri *et al*., 2019; Bancalari *et al*., 2020; Attia *et al*., 2023**). Application of *A. platensis* could induce resistance against Fusarium wilt disease in pepper, which resulted in a significant increase in the expression of all metabolic resistance indices; phenols, polyphenol oxidase, and peroxidase (**Attia *et al*., 2023)**. Moreover, *B. cinerea* was hindered by phycobiliproteins isolated from *A. platensis*, which prevented spore germination as well as mycelial growth and development. (**Righini *et al*., 2020**). In a small-scale trial , polysaccharides and phycobiliproteins of *A. platensis*, administered prior to *Botrytis cinerea* inoculation, suppressed the causative pathogen on tomato fruit. The secondary structure of α-helix, which is present in transmembrane proteins, was identified through the characterization of polysaccharides and phycobiliproteins. This structure is likely responsible for disrupting the function of fungal cells (**Righini *et al*., 2020**). *A. platensis* polysaccharides caused a variety of biochemical changes involved in the construction of wax and cutin in tomato leaves, including fatty acids (C16:3, C18:2, and C18:3, C18:0), alkanes (eicosane, octacosane, tetracosane, nonacosaene, triacontane, and dotriacontane; the alkane derivate 1-chloroeicosane) and alkenes (1-octadecene, 1-pentadecene). These biochemical changes were linked to plant defense compounds, phenylalanine ammonia-lyase (PAL), chitinases, glucanases, and peroxidases activities, as well as H_2O_2 accumulation. The same leaves also included other novel metabolites, including tris(2,4-di-tert-butylphenyl) phosphate, alpha-tocospiro-B, neophytadiene, and (2(4H)-benzofuranone (**Rachidi *et al*., 2021**; **Righini *et al*., 2022)**.

It is noteworthy to mention that nostocales have been extensively studied since the early 2000s. However, *Nostoc* spp. suppressed mycelial growth of

Aspergillus spp. in agar disk diffusion assay (**El-Sheekh *et al*. 2006; Pawar *et al*. 2008; Shishido *et al*. 2015**). Reduction in growth of *Fusarium* species was reported with *Nostoc* spp., including *N. muscorum* (**El-Sheekh *et al*. 2006; Kim and Kim 2008**). Methanol extract of *N. muscorum* reduced *A. alternata* (**Kim 2006**). Phenols and polysaccharides extracted from *Nostoc* spp., were involved in the antifungal activity against *R. solani* (**Ismail and Ismail, 2011**). A phenolic compound isolated from the chloroform extract of *N. muscorum* showed a strong activity against *Aspergillus niger*, *A. flavus*, *Pencillium* sp., and *F. microsporium* (**El-Sheekh *et al*., 2006**). *N. muscorum* has various important enzymes such as; peroxidase, hydrogenase, and alkaline phosphatase used for the biotransformation of hydrocortisone as an exogenous substrate (**Asakawa and Noma, 2010**). It is noteworthy that these biological inducers are known to have some eliciting activities, leading to a variety of defense and/or resistance reactions or mechanisms in the host plants, in response to microbial infection. The main principal defense-related mechanisms include the accumulation of phenolic compounds, and pathogenesis-related (PR-proteins) (**Nafie and Mazen, 2008**) and enhancement of the activity of defense-related enzymes (**Govindappa *et al*., 2010**).

Further studies are very needed in the future, to accurately investigate and comprehensively explore the important role of these biocontrol agents and others in enhancement and increase a host resistance against rust pathogens, including yellow rust in wheat, as the dangerous fungal pathogens threaten wheat production in Egypt and worldwide. Furthermore, these important researches should facilitate the beneficial use and good utilization of the tested biocontrol agents, as useful alternatives to the wide application of the synthetic fungicides.

CONCLUSION

Application of the six biocontrol agents to induce yellow rust resistance in wheat plants (cv. Gemmeiza-11), *i.e.*, *P. fluorescens* (strain OR485143), *A. platensis*, *N. muscorum*, 8-Quinolinol, cinnamic acid and fulvic acid, significantly decreased host response as well as average coefficient of infection on adult plants, and subsequently increased grain yield components. Each of them offers a good level of disease protection through the induction and accumulation of antifungal substances such as a total phenolic content, and increasing enzymatic activity of POX and PPO enzymes, involved in metabolic defense-related mechanisms. Such treatments can be recommended as eco-friendly tools to integrate disease management for further field crops, especially wheat. The use of alternative bio-products in place of synthetic fungicides for plant disease control is also previously encouraged and/or suggested by European Directive 2009/128/EC. The current study provides useful results and profitable information on the use of the tested biological inducers under study for their substantial bioprotective effect against wheat yellow rust, and their capability to induce some plant defense mechanisms.

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