Asian Journal of Research in Crop Science

Volume 9, Issue 2, Page 156-167, 2024; Article no.AJRCS.117205
ISSN: 2581-7167

Biopotential of *Cyanobacteria*, Fulvic Acid and Nanochitosan in Controlling Leaf Rust of Wheat

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ajrcs/2024/v9i2276

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/117205

Original Research Article

Received: 18/03/2024
Accepted: 20/05/2024
Published: 05/07/2024

ABSTRACT

Biocontrol agents, including *Cyanobacteria*, *Spirulina platensis* and *Nostoc calcicole*, as well as fulvic acid and chitosan nanoparticles were tested to control leaf rust of wheat under field conditions during 2021/2022 and 2022/2023 growing seasons. All biocontrol agents significantly reduced all parameters for disease severity *i.e.* coefficient of infection (CI), the average coefficient of infection (ACI) and the area under disease progress curve (AUDPC) compared to untreated control. The best treatments were nano-chitosan which recorded efficacy of 91.17% followed by *S. platensis* (90.2%) in the application of two sprays for disease control. All biocontrol agents significantly increased grain yield components of wheat. Applications of two sprays were more effective than one spray. From our research work it can be concluded that *S. platensis*, *N. calcicole*, fulvic acid and nano-chitosan can be used for controlling leaf rust disease as a safe alternative to chemical fungicides.

Keywords: Biological control; wheat; leaf rust; cyanobacteria; fulvic acid; nano-chitosan.

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Cite as: *Elkhwaga, Amal A., Hala A. Eldakar, Hesham M. Abdelmaksoud, Noha E. Ahmed, and Mohammed A. Gad. 2024. "Biopotential of Cyanobacteria, Fulvic Acid and Nanochitosan in Controlling Leaf Rust of Wheat". Asian Journal of Research in Crop Science 9 (2):156-67. https://doi.org/10.9734/ajrcs/2024/v9i2276.*

1. INTRODUCTION

"Wheat crop is affected by various biotic agents. Among the biotic agents, yellow or stripe rust caused by *Puccinia striiformis* f. sp. *tritici*, and leaf rust caused by *Puccinia triticina* f. sp. *tritici*, that cause severe losses in yield components because their wide distribution, their capacity to form new races and their capability to disseminate to long sites" [1,2,3,4,5,6,7,8,9,10, 11,12,13,14], (Gad et al. 2021). "Leaf rust is one of the most important common diseases which attack leaves of wheat (*Triticum aestivum* L.)" [15]. "Leaf rust can reduce grain yield of wheat by 35–50%" [16].

"Generally, rust control by fungicide application is one of the most popular means of maximizing grain yield globally. Fungicides can also play a role in an integrated management of the disease until new cultivars with genetic resistance are available" [17]. "However, the excessive and irrational use of synthetic fungicides has perturbed us with irrevocable soil-water-air contaminations, development of resistance in microbes, and disturbing biosphere" [18]. "Recently using biocontrol had gained considerable attention as alternative options to synthetic fungicides and efforts have been made to utilize, the biocontrol has strategies against plant diseases" [19].

"*Cyanobacteria*, also called blue-green algae produce a wide range of bioactive compounds that are mostly used in cosmetics, animal feed, human food, nutraceutical and pharmaceutical industries, and the production of biofuels. The research concerning the use of *Cyanobacteria* in agriculture has pointed out their potential as a source of bioactive compounds, such as phycobiliproteins, for plant pathogen control and as inducers of plant systemic resistance" [20]. *Arthrospira* (Spirulina) *platensis* is one of the photoautotrophic, planktonic, filamentous green-blue algae (Cyanobacterium) that have become of medical interest [21]. *S. platensis* extract contains phenolics that resulted in their antifungal activity [22,23,24]. "Spirulina was found to act as a probiotic and antioxidant agent" [25,26,27] "*S. platensis* contains high protein levels with all essential amino acids, essential fatty acids, minerals, pigments, carotenoids, and vitamins" [25,28]. Nostocales have been extensively studied since the early 2000. The microalgae (*Nostoc calcicole*) are more primitive than terrestrial plants and they are capable of producing relatively complex polyphenols [29]. Alkaloids are commonly found to have antimicrobial properties [30] against both Gram-positive and Gram-negative bacteria [31]. Extract of *N.calcicola* HN9 expressed positive effect on development, growth and raised soybean productivity.

"Nanochitosan or chitosan nanoparticles having biocompatibility, biodegradability, wide biological activities, and ecological safety characteristics are in the forefront list of scientists" [32,33,34]. Potential of nano-chitosan as a biocontrol agent against many plant diseases, including wheat rusts has been previously reported [35,36]. Fulvic acid is a plant bio-stimulant that is produced basically by bio-degradation of lignin containing plant organic matter [37]. Fulvic acid has been early recorded to have an appositive effect against plant pathogens [38,39].

The present study investigated the antifungal efficacy of *Cyanobacteria* (*Spirulina platensis, Nostoc calcicole*), fulvic acid and chitosan nanoparticles against wheat leaf rust and to determine the histopathologic defence mechanisms involved control of the rust disease.

2. MATERIALS AND METHODS

2.1 Preparation of *Cyanobacteria*

Filamentous heterocystous *Cyanobacteria* or blue-green algae (*Spirulina platensis* and *Nostoc calcicole*) were obtained from Soil, Water and Environmental Institute, ARC, Egypt. *S. platensis* was maintained in Zarrouk's liquid medium [40], *N. calcicole was* maintained in BG-11 liquid medium [41]. "Cultures were separately maintained in 1 L flasks with 300 mL culture medium composed of sterilized tab water. Each Flask was inoculated with 3 ml of *Cyanobacteria* were studied, incubated at 29 ± 2°C under a 12h-light/12h-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}. Manual shaking of cultures was done 3–4 times daily" [42]. "After 20 days, the biomass from the cultural medium was separated by filtration of culture media by filter paper Whatman No.1, and then using the filtrate output as treatment" [43]. The extracts were adjusted to a final concentration of 100 ml L^{-1}.

2.2 Preparation of Fulvic acid

Fulvic acid (FA) was obtained from Technogen Chemical Co., Egypt, and prepared according to the methods described by Kononova [44].

2.3 Preparation of Chitosan Nanoparticles

Synthesis and characterisation of chitosan nanoparticles were described in our previous study of ElKhwaga et al. [36] and used in the current study. The used concentration of nano-chitosan was 150 µg mL^{-1} (150 ppm).

2.4 Biological Control of Wheat Leaf Rust

The Experimental was conducted at El-Gemeiza Agricultural Research Station, ARC, Gharbiya, Egypt during two cropping seasons 2021/2022 and 2022/2023. Biological treatments as, *Spirulina platensis*, *Nostoc calcicole*, fulvic acid and chitosan nanoparticles were tested for their biopotential efficacy against wheat leaf rust disease under field conditions. A split plot design with three replicates was used to set up the experiment. Seeds of susceptible wheat variety "Gemmiza-7" were sown in plots consisted of three rows with 3 m long and 30 cm apart received 15 g of seeds/row. The main plots were represented by one- and two-sprays for each treatment. The sub-plots were represented by the tested treatments and all the plots were surrounded by a spreader area with a highly susceptible wheat cultivar "Morocco" and all cultural practices recommended in the commercial fields were applied. The artificial inoculation was carried out at the 7^{th} growth stages [45] by using a mixture of urediniospores of *Pt* isolates according to Tervet and Cassel [46]. The biological treatments were applied twice at 7-8^{th} growth stage [45]. The first spray was applied 1 day before pathogen inoculation and the second spray was applied at the disease appearance (3%) (About 15 days of the first one). The fungicide (propiconazole 25%) at a rate of 0.25 ml L^{-1} was used for comparison. The untreated control were sprayed with distilled water.

2.5 Disease Assessment

Leaf rust scoring was quantified based on the coefficient of infection (CI) according to Saari and Wilcoxson [47] and the area under disease progress curve (AUDPC) according to Pandey et al. [48]. Rust scoring was assessed four times at growth stages GS 51 to GS 83, at 10 days intervals [49]. The infection types were scored according to Roelfs et al. [50] as resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible (S). Rust severity was evaluated using Modified Cobb's scale [51]. The coefficient of infection (CI) was calculated by multiplying the severity value by a constant value of 0.2, 0.4, 0.6, 0.8 and 1.0 for infection types ratings of R, MR, MR-MS, MS and S, respectively. The average coefficient of infection (ACI) was calculated as mean CI values over seasons. The AUDPC was calculated using the following equation:

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

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

The efficacy % of treatments was estimated according to Rewal and Jhooty [52]: using the following equation:

$$Efficacy = c\text{-}t/c \times 100$$

where c = infection in control, while t = infection in treatment.

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

2.6 Scanning Electron Microscope (SEM)

Leaf specimens of leaf rust infected wheat and other treated with the tested biological agents, were harvested using sterilized scissors at 1 day post inoculation (dpi) for scanning electron microscopy investigation. Sample preparation for SEM examination was carried out as described by Harley and Fergusen [53]. The SME assay was carried out using a Jeol scanning electron microscope at the National Research Center, Egypt. Ultra-structural changes on the urediniospores of *P. triticina* were investigated in treatments and untreated control.

2.7 Statistical Analysis

Data were subjected to the analysis of variance (ANOVA) using the SAS Statistical Analysis System package v.22 (SAS Institute, Cary, NC, US). Means of data were separated at the least significant difference (LSD) test at $P \leq 0.05$ [**54]**.

3. RESULTS

3.1 Potential of Biocontrol Agents to Control Wheat Leaf Rust

Biopotential of *Cyanobacteria*, fulvic acid and nano-chitosan to control leaf rust of wheat (cv. Gemmeiza-7) was tested under field conditions

during 2021/22 and 2022/23 growing seasons. Data in Table (1) showed significant differences in leaf rust assessments CI, ACI and AUDPC between treatments, sprays and seasons, while interactions between them were insignificant.

Leaf rust assessments CI, ACI and AUDPC were significantly reduced due to biological treatments compared to untreated control. Application of two sprays was better than one spray for all treatments showing high significant differences. In the calculated means for biological treatments, the lowest values of ACI and AUDPC were recorded with nano-chitosan which recorded ACI values of 14.33 and 7.5, and AUDPC values of 122 and 61.83 for one and two sprays, respectively. Cyanobacterium *S. platensis* ranked the second with ACI of 17 and 8.33, and AUDPC of 131.66 and 65.66, followed by fulvic acid with ACI of 24.33 and 8.33, and AUDPC of 192.83 and 67.66 for one and two sprays, respectively. The aforementioned treatments were comparable to fungicide (propiconazole 25%) which recorded ACI of 13.66 and 5,33, and AUDPC of 91.33 and 50.33 for one and two sprays, respectively. Cyanobacterium *N. calcicole* also significantly reduced ACI to 25 and 16.66, and AUDPC to 204.16 and 121.83 for one and two sprays, respectively, as compared to untreated control with ACI of 85 and AUDPC of 915.

3.2 Efficacy of Biocontrol Agents Against Wheat Leaf Rust

Fig. (1) showed that all the tested biocontrol agents were effective to control leaf rust where the efficiency ranged from 70.58 to 83.14% and 80.04 to 91.17% for one and two spray, respectively. Nano-chitosan was the most effective treatment (83.14, 91.17%), followed by cyanobacterium; *S. platensis* (80, 90.2%), and fulvic acid (71.37, 90.2%) for one and two sprays, respectively. The aforementioned treatments were comparable to fungicide (propiconazole 25%) which recorded 84.31 and 93.72% efficacy. Application of *N. calcicole* also showed efficacy of 70.58 and 80.4% for one and two sprays, respectively.

3.3 Effect of Biocontrol Agents on Grain Yield Components

Data in Tables (2 and 3) showed that all biocontrol agents significantly increased the grain yield components in terms of spike weight, 1000-kernel weight, and volume weight in comparison to the untreated control during both growing seasons 2021/22 and 2022/23. High significant differences were recorded between the treatments and untreated control. Nanochitosan recorded the highest weights of grain yield components followed by *S. platensis* and fulvic

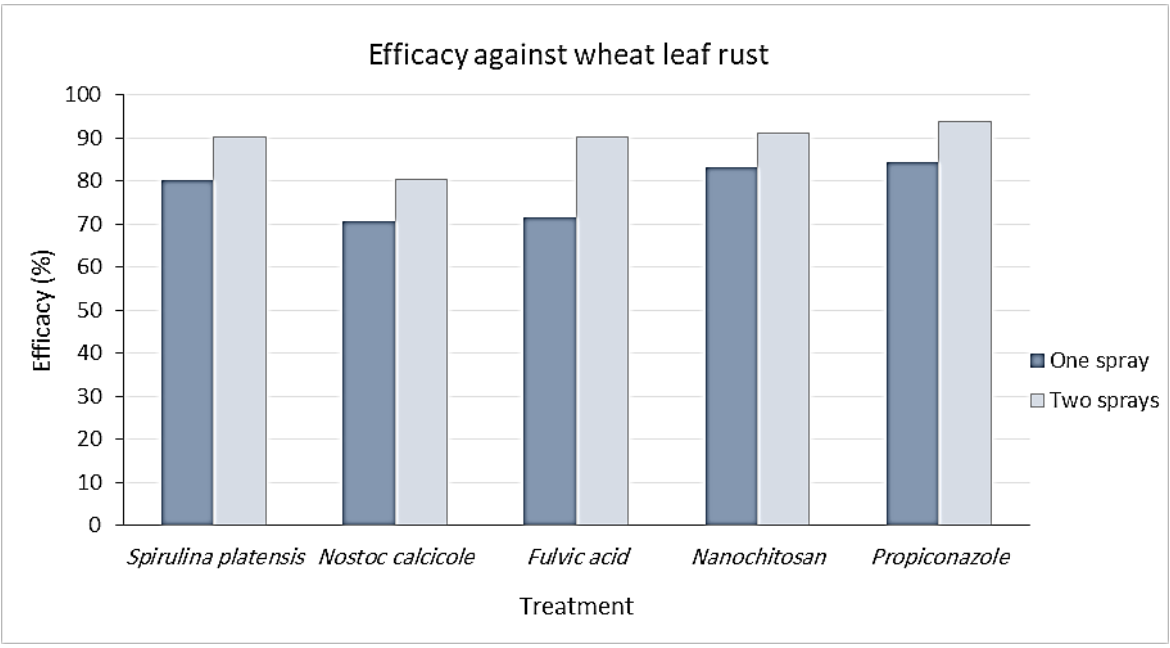

Fig. 1. Efficacy of *Cyanobacteria*, fulvic acid and nano-chitosan to control leaf rust of wheat (cv. Gemmeiza-7) under field conditions

Table 1. Biopotential of *Cyanobacteria*, fulvic acid and nano-chitosan to control leaf rust of wheat (cv. Gemmeiza-7) under field conditions during 2021/22 and 2022/23 growing seasons

Treatment	CI				ACI		AUDPC				Mean AUDPC	
	1st season		2nd season				1st season		2nd season			
	Sp[1]	Sp[2]	Sp[1]	Sp[2]	Sp[1]	Sp[2]	Sp[1]	Sp[2]	Sp[1]	Sp[2]	Sp[1]	Sp[2]
Spirulina platensis	17.33	8.33	16.33	8.33	17	8.33	134	67.66	129.33	63.66	131.66	65.66
Nostoc calcicole	26.66	16.66	23.33	16.66	25	16.66	205	124	203.33	119.66	204.16	121.83
Fulvic acid	26.66	8.33	22	8.33	24.33	8.33	195.66	70.33	190	65	192.83	67.66
Nanochitosan	15.33	8.33	13.33	6.66	14.33	7.5	124	64	120	59.66	122	61.83
Propiconazole	14	5.33	13.33	5.33	13.66	5.33	94.33	52	88.33	48	91.33	50.33
Control	86.66		83.33		85		980		850		915	
$LSD_{0.05\ Treatment\ (T)}$	6.34		6.16		5.86		7.62		6.14		6.7	
$LSD_{0.05\ Spray\ (Sp)}$	4.12		4		1.63		2.41		2.32		0.94	
$LSD_{0.05\ Season\ (S)}$					2.18						2.5	
$LSD_{0.05\ T \times S}$					Ns						ns	
$LSD_{0.05\ T \times S \times Sp}$					Ns						ns	

CI = coefficient of infection, ACI = average coefficient of infection (ACI), AUDPC = area under disease progress curve, r-AUDPC = relative AUDPC, Sp[1] = one spray, Sp[2] = two sprays

Table 2. Grain yield components of wheat (cv. Gemmeiza-7) treated with *Cyanobacteria*, fulvic acid and nanochitosan against leaf rust under field conditions during 2021/22 growing season

Treatment	Spike weight (g)		1000-kernel weight (g)		Volume weight g L^{-1}	
	Sp[1]	Sp[2]	Sp[1]	Sp[2]	Sp[1]	Sp[2]
Spirulina platensis	3.85	3.95	50.6	54.20	689.56	695.14
Nostoc calcicole	3.77	3.87	46.66	49.80	675.45	682.57
Fulvic acid	3.90	4.00	50.42	55.15	690.52	702.11
Nanochitosan	4.13	4.42	57.36	62.42	710.53	721.54
Propiconazole	4.10	4.35	55.64	61.28	710.50	718.22
Control	3.05		40.45		630.11	
$LSD_{0.05\ Treatment\ (T)}$	0.16		1.2		5.62	
$LSD_{0.05\ Spray\ (Sp)}$	0.12		0.84		3.15	
$LSD_{0.05\ T \times Sp}$	0.8		Ns		ns	

Sp[1] = one spray, Sp[2] = two sprays

Table 3. Grain yield components of wheat (cv. Gemmeiza-7) treated with *Cyanobacteria*, fulvic acid and nanochitosan against leaf rust under field conditions during 2022/23 growing season

Treatment	Spike weight (g)		1000-kernel weight (g)		Volume weight g L^{-1}	
	Sp^1	Sp^2	Sp^1	Sp^2	Sp^1	Sp^2
Spirulina platensis	3.87	3.98	50.61	54.32	689.56	695.14
Nostoc calcicole	3.80	3.88	46.68	49.85	675.45	684.21
Fulvic acid	3.93	4.02	50.64	55.35	690.52	702.11
Nanochitosan	4.18	4.50	59.41	62.78	711.33	722.42
Propiconazole	4.15	4.37	55.75	61.60	710.78	719.54
Control	3.17		41.53		632.24	
$LSD_{0.05}$ Treatment (T)	0.21		1.06		4.97	
$LSD_{0.05}$ Spray (Sp)	0.18		0.92		3.23	
$LSD_{0.05}$ T × Sp	0.65		Ns		ns	

Sp^1 = one spray, Sp^2 = two sprays

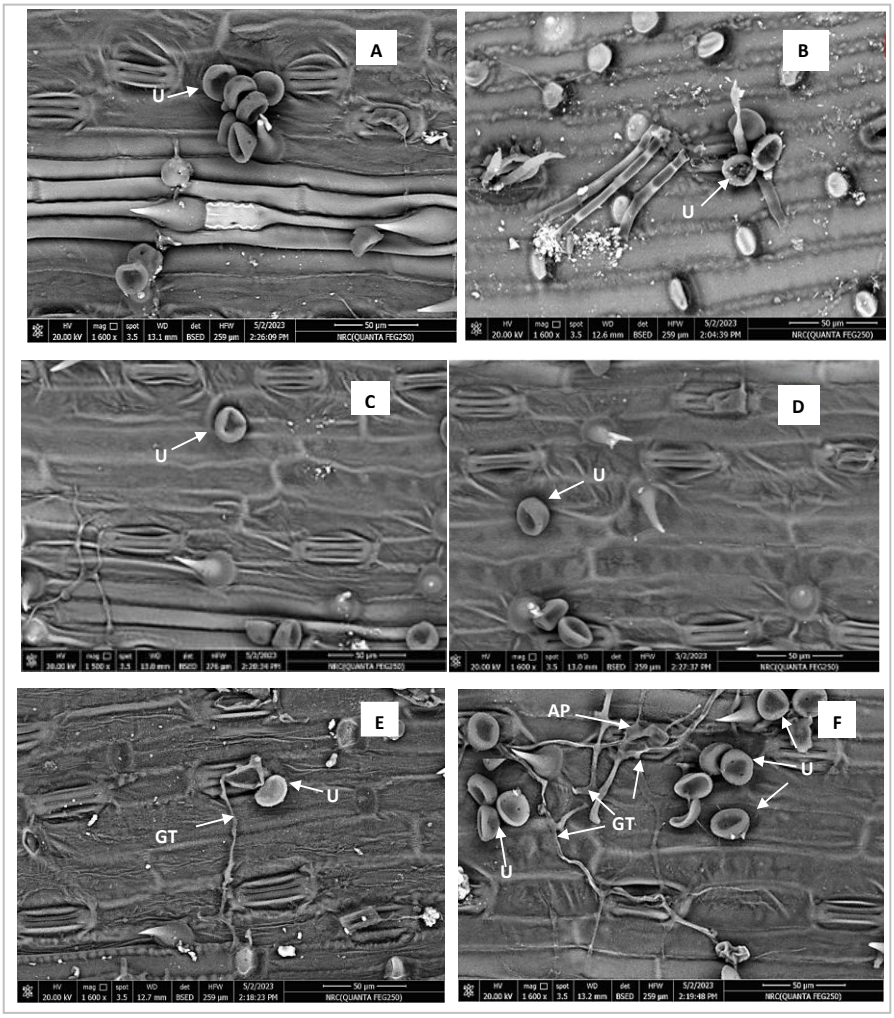

Fig. 2. SEM observation of *P. triticina* urediniospores on adult plant leaves of wheat (Gemmeiza-7) treated with biological agents, *Spirulina platensis* (A), *Nostoc calcicole* (B), Fulvic acid (C), nanochitosan (D), Fungicide propiconazol 25% (E), and untreated control (F). U: Urediniospore, GT: Germ tube, AP:appressorium.

acid during both seasons. Data in the first season (Table 2) showed that nanochitosan recorded spike weight of 4.13, 4.42 g and 1000-kernel weight of 57.36, 62.42 g, volume weight of

710.53, 721.54 g L^{-1}, followed by cyanobacterium *S. platensis* 3.85, 3.95 g spike weight, 50.6, 54.20 g 1000-kernel weight, and 689.56, 695.14 g L^{-1} volume weight for one and two sprays, respectively. Data in the second season (Table 3) showed that nanochitosan recorded 4.18, 4.50 g spike weight and 59.41, 62.78 g 1000-kernel weight and 711.33,722.42 g L^{-1} volume weight, followed by cyanobacterium *S. platensis* with 3.87, 3.98 g spike weight, 50.61, 54.32 g 1000-kernel weight and 689.56, 695.14 g L^{-1} volume weight for one and two sprays, respectively. The aforementioned treatments were comparable to fungicide propiconazole 25% during both seasons. Grain yield components associated with application of *N. calcicole* were also significantly increased during both seasons as compared to untreated control.

3.4 Scanning Electron Microscope (SEM)

Fig. (2) illustrated urediniospore observation using SEM of urediniospores of *P. triticina* on adult plant leaves of wheat (Gemmeiza-7) treated with biological agents, *Spirulina platensis, Nostoc calcicole,* fulvic acid and nano-chitosan, as compared to fungicide propiconazol 25% and untreated control. All biological agents inhibited spore germination at different levels, while abundant germination was observed in untreated control. Fungicide (propiconazol 25%) also reduced spore germination. The non-germinated urediniospores were observed to be shrivelled by application of *S. platensis* and *N. calcicole,* (Figs. 2, A, B). Change in shape of non-germinated urediospores from round to oval were observed by application of fulvic acid and nano-chitosan (Figs. 2, C, D). Swelling of germinated urediniospores were observed by application of fungicide propiconazole 25% (Fig. 2, E). In untreated control, normal shape of germinated urediniospores and germ tubes were observed along with appressorium formation (Fig. 2, F).

4. DISCUSSION

“Eco-friendly control of plant diseases using biological agents which act directly on the plant pathogens in plants, have gained considerable attention as alternative means to synthetic fungicides” [38,35]. “Using natural biological material in the control of wheat rusts is a modern, advanced and risk-free alternative method of rust management” [55]. “*Cyanobacteria*, *Spirulina platensis* and *Nostoc calcicole* as well as fulvic acid and nanochitosan, are among the biological control agents that directly affect the phytopathogens” [37,56,25,35]. In current study, we tested the biopotential of *S. platensis, N. calcicole*, fulvic acid and nano-chitosan to control leaf rust of wheat (cv. Gemmeiza-7) during two growing seasons. All biocontrol agents tested significantly reduced the CI, ACI and AUDPC as compared to fungicide (propiconazole 25%) and untreated control. The best treatment was nanochitosan, followed by cyanobacterium *S. platensis* and fulvic acid. Application of *N. calcicole* also significantly reduced CI, ACI and AUDPC as compared to untreated control. The tested biocontrol agents showed high efficacy in controlling wheat leaf rust which reached 91.17% in nanochitosan, followed by *S. platensis* and fulvic acid (90.2% each) for two sprays. Efficacy of *N. calcicole* against wheat leaf rust was 80.4% for two sprays.

Chitosan nanoparticles reduce spore germination and increase latency and periods of incubation meanwhile, decrease the type of infection, size, and pustules number compared to the untreated control [35]. Chitosan nanoparticles have a potent antimicrobial effect due to their ability to bind microbial proteins and produce cell membrane permeability and disintegration [57]. Chitosan effects on hyphal development of plant pathogens [58]. “In pursuit of this, chitosan, β-(1,4)-2-amino-2-deoxy-d-glucose, a hetero-aminopolysaccharide which can easily be obtained from the waste produce of shrimp, crab shells, and cell wall of fungi” [59,60]. “In anatomical examinations, chitosan nanoparticles enhanced thickness of blade (µ), thickness of mesophyll tissue, thickness of the lower and upper epidermis and bundle length and width in the midrib compared to the control. In the control treatment's top epidermis, several sori and a large number of urediniospores were found. Nanochitosan has been previously proven to induce resistance against leaf rust in Egypt” [36,35]. “Fulvic acid is plant biostimulant that are produced mainly by biodegradation of lignin containing plant organic matter. Application of fulvic acid to plants affects cell membranes, leading to enhanced transport of minerals, improved protein synthesis, plant hormone like activity, promoted photosynthesis, modified enzyme activities, solubilization of micro and macro elements, reduction of active levels of toxic minerals, and increased microbial populations” [61]. “In general, fulvic acid substances are a suspension based on potassium humates which can be applied successfully in many areas of plant production as a plant growth stimulant or soil conditioner for

enhancing natural resistance against plant diseases and pests" [62,63]. "Foliar application of fulvic acid improved plant growth and yield quantity and quality as well as controlling powdery and downy mildews of cucumber plants" [38]. Fulvic acid has the advantage as effective and environmentally friendly agent.

"The use of *Spirulina*-based stimulators is reliable with the concept of sustainable agriculture by enhancing photosynthetic pigment content and rate ensuring the correlation between the yield and those measured parameters. *Spirulina platensis* extract contains Phenols that resulted in their antifungal activity" [22,23,24]. "The promotion of growth could be attributed to the nutrients, bioactive molecules and phytohormones in the *Spirulina* extract" [64]. Nostocales have been extensively studied since the early 2000. *Nostoc* spp. inhibited *Aspergillus* spp. mycelial growth in agar disk diffusion assay [65,66,67,68]. Reduction in growth of *Fusarium* species was reported with *Nostoc* spp. [66,69,70]. "Phenols and polysaccharides contained in extracts from *Nostoc* spp. are involved in the antifungal activity against *R. solani"* [71]. In the current study, scanning electron microscope showed that all biological agents inhibited spore germination, while abundant germination was observed in untreated control. Fungicide (propiconazol 25%) also reduced spore germination.

In the current study these materials showed efficacy in controlling the leaf rust disease of wheat that was comparable to fungicide (propiconazole 25%) and also significantly enhanced the grain yield components.

5. CONCLUSION

Application of *Cyanobacteria*, *Spirulina platensis* and *Nostoc calcicole* as well as fulvic acid and nanochitosan, as biological control agents showed directly effect on wheat leaf rust. They had the efficacy to reduce CI, ACI and AUDPC of leaf rust disease under field conditions. They also significantly increased grain yield components of wheat. Our study indicated that the tested bio-control agents could be used for the control of leaf rust disease of wheat as a safe alternative to chemical fungicides.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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