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ORIGINAL PAPER

Alternative Management of Wheat Leaf Rust Caused by *Puccinia triticina* Revealing Histological and Biochemical Defense Mechanisms

Mabrouk, O.I .[1]; Draz, I. S.[1]; Farouk, A.M.F [3]; Omar, G. E[2]; Najeeb, K.M.A.[1] and Zayton, M.A[3].

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ABSTRACT

Worldwide wheat production is seriously threatened due to infection by wheat leaf rust caused by *Puccinia triticina*. This study assessed the effectiveness of four tested treatments e.g. Bio-Arc, Bion, Frankincense oil and Cyanobacteria against the wheat leaf rust under greenhouse conditions on seedling and adult stages, as well as the mechanisms underlying disease resistance. Compared to other treatments, the application of Bion and Frankincense oil inhibited spore germination and increased incubation and latent periods. Moreover, had the least infection type, pustule size, and the number of pustules cm^2, decreased the incidence of leaf rust infection (final rust severity%, rate of disease increase, and area under disease progress curve) and was effective up to 73.85 and 88.9% at the seedling and adult stage, respectively, compared to the untreated control. Bio-Arc's treatment had a moderate efficacy of 50 and 47.23%, respectively. While, Cyanobacteria was the least one in their effect. Using scanning electron microscope; abnormalities were observed in treated leaves, including urediniospore lysis, collapse, and shrinkage in four tested treatments. Treatments led to a significant increase in the activities of antioxidant defense enzymes, specifically catalase (CAT), peroxidase (POX), and polyphenol oxidase (PPO). GC-MS analysis used with the treated wheat leaves by four treatments, identified 41 compounds that were identified at different rotation times as the active organic compounds.

Keywords: Enzymes activities, Bio-materials, *Puccinia triticina*, SEM, GC-MS.

***Correspondence: Ola I. Mabrouk**
E-mail: ola_pathology@yahoo.com

Ola I. Mabrouk
https://www.orcid.org/0000-0002-5715-6714
Ibrahim S. Draz
https://orcid.org/0000-0002-2581-3556
Khadegah M.A. Najeeb
https://orcid.org/0009-0005-1987-2700
1-Wheat Diseases Research Department, Plant Pathology Research Institute, Agricultural Research Center, 12619, Giza, Egypt.
Ghady E. Omar
https://orcid.org/0009-0003-8566-3670
2-Seeds Pathology Research Department, Plant Pathology Research Institute, Agricultural Research Center, 12619, Giza, Egypt.

Amany M. Farouk
https://www.orcid.org/0000-0002-0075-3438
Marwa A. Zayton
https://orcid.org/0009-0005-9835-920X
3-Plant Pathology Department, Faculty of Agriculture, Cairo University

INTRODUCTION

In Egypt, among other diseases, wheat is susceptible to infection by rusts, powdery mildew and smuts Under Egyptian

conditions, wheat rusts continue to be the primary biotic stressor that impacts the quantity and quality of wheat cultivars yields (**Mabrouk *et al.*, 2022).** stem rust (*Puccinia tritici*) is considered one of the three primary rusts that harm wheat plants in Egypt, however the infection by the leaf rust (*Puccinia triticina)* consider as a big obstacle to wheat production in Egypt, in addition to the cultivars susceptibility to environmental conditions leads to significant yield losses that could exceed 20% **(Soliman *et al*., 2016).** The absence of resistance in various wheat varieties increases the severity of the disease, and an outbreak may happen quickly due to ongoing genetic changes in host-pathogen interactions and the emergence of new aggressive leaf rust races that have the potential to replace wheat cultivars' resistance **(Omara *et al.*, 2021 and Atia *et al.*, 2021).** Although a large number of fungicides are able to controlling diseases, their long residual effects and potential for exposure pose a risk to human health and safety **(Berny 2007; Aktar *et al.*, 2009 and Thabet *et al.*, 2023)**. Nevertheless, the fungus demonstrates a high degree of adaptability by growing outbreaks resistant to fungicides and conquering plant resistance **(Cowger *et al*., 2000; Cheval *et al.*, 2017).** So, trend to assess eco-friendly control methods like organic materials and plant extracts, which either directly attack plant diseases, or indirectly by conferring resistance on plants **(Mishra *et al.*, 1999),** that have drawn significant attention as viable substitutes for synthetic fungicides, Plant extracts have been used in attempts to control plant diseases (**Srivasata *et al*., 2011, Elsharkawy & El-Sawy, 2015, Ahmed *et al.*, 2019 and Mohdly *et al*., 2024)**.

Bio-Arc 6% (w/w) is a commercial bio product with active ingredients *Bacillus megaterium*, Recent studies have identified *Bacillus* species as potential biocontrol agents and enhancers of plant growth across a wide range of plant species. *Bacillus* species produce at least 66 distinct antibiotic compounds that contribute to the biological management of plant disorders and enhance plant development **(Ferreira *et al*.,1991)**. While Benzothiadiazole (BTH or Bion) has been demonstrated to confer resistance against plant pathogens, offering three primary benefits: a wide range of effectiveness and minimal environmental repercussions. Moreover, the likelihood of pathogen strains developing resistance to Bion is negligible**. (Lawton *et al.*, 1996 and El-Habbak, 2022).** Frankincense is a resinous substance derived from the Boswellia tree (*Boswellia carterii* Flueck) by making a large, longitudinal cut across its trunk. In all, nine compounds with immune-stimulating and antiviral qualities were found in Frankincense oleo-gum resin. many investigators concluded that the essential oil of frankincense contains monoterpenes, sesquiterpenes, and diterpenes, oil demonstrating potent immune stimulant effects **(El-Nagar *et al.*, 2022 and Al-Kharousi *et al.*, 2023)** Eugenol is a potent ingredient, which has been effectively utilized to eradicate numerous plant pathogens by preventing *P. striiformis* uredospores from germination and reducing latent and incubation times during the seedling stage, eugenol treatment decreased rust severity (%) and AUDPC at the adult stage **(El-Sawy *et al.*, 2016).** For instance, the severity of stag

head infection and white rust disease caused by *Albugo candida* was significantly reduced by sprays of a 0.02% solution of Bion 50WG (*bezothiadiazole*) **(Kumar, 2009)**, and examine how essential oils affect the growth of mycelium and the inhibition of spore germination. Seven economically significant rice pathogenic fungal species were treated with frankincense oil which is derived from the (*Boswellia carteri bird*). These fungi are *Aspergillus flavus, Bipolaris oryzae, Alternaria brassicicola, Fusarium moniliforme, Pyricularia arisea,, Rhizoctonia solani and Fusarium proliferatum.* The concentration of 2.0% v/v of frankincense oil showed the greatest inhibition of spore germination and mycelium growth, **(Julara*t et al.,* 2009).** Cyanobacteria are commonly referred to as "blue-green algae," which is a convenient term for water-dwelling creatures that produce their own food.

Cyanobacteria are connected to bacteria, not eukaryotes, some Cyanobacteria are aquatic and photosynthetic, meaning they live in water and produce their own food, (**Issa *et al.,* 2014**). The use of Cyanobacteria in agriculture is considered as bio-agents to sound crop management methods such as the use of minimal tillage, organic manures, crop rotation and the biocontrol of diseases and pests **(Higa and Wididana, 1991).** Meanwhile certain species of Cyanobacteria have an antagonistic effect on plant pathogens, as *Nostoc muscorum* against *Rhizoctonia solani*'s damping off and *Sclerotinia sclerotiorum's* cottony rot of flowers and vegetables **(De Caire *et al.,* 1990; Kulik, 1995 and Tassara *et al.,* 2008)** *Calothrix elenkenii* against *Rhizoctonia solani* damping off (**Manjunath *et al.,* 2009)** *Muscicola Fischerella* with Rice blast *(Pyricularia oryzae)* and powdery mildew *(Erysiphe graminis)* **(Hagmann and Juttner, 1996).**

Biological control appears to be the most promising among the options for environmentally friendly and sustainable agriculture to preserve food and crop. **(Syed *et al.,* 2018),** although, current data does not support the notion that the use of biological control could enhance agricultural productivity and disease management in a way that is more convenient, effective, and profitable **(Zhan *et al.,* 2014).** Biological invasion can be caused by intricate interactions between physical environments, BCAs, pathogens, and crop plants, putting the local ecosystem in jeopardy and raising the possibility that, if applied in a single, static way over an extended period of time, the efficacy of biological control techniques will be eroded or lost entirely **(Dun-Chun *et al.,* 2021).** This emphasizes how it is crucial to use the ecological evolutionary principle in concert with other factors to assess the effectiveness, efficiency, longevity, and environmental safety of biological control. Therefore, this study examined the efficacy of a few bio treatments in vitro and in greenhouse settings as a means of managing wheat leaf rust.

MATERIALS AND METHODS

Source of the tested materials

Four materials were used *i.e.*, the commercial bio product Bio-Arc 6% with active ingredient *Bacillus megaterium* with recommended dose 2.5 g L^{-1},

Benzothiadiazole (BTH or Bion) is a chemical activator for disease resistance in various plants with 1mM concentration, the natural substance Frankincense essential oil (*Boswellia carterii* Flueck.) with 5% concentration and "blue-green algae, (Cyanobacteria) biostimulator (0.1%), a mixture of different blue-green algae species provided by Soil Water & Environ. Res. Inst., ARC . Water was used as an untreated control while check control was treated with 0.1% concentration pilizol (25% propiconazole EC 200 cm^3 $100L^{-1}$ water) to serve as protected control against leaf rust infection .

Microscopic examinations

Examination of uredospores germination

To study the effect of the tested treatments on germination of uredospores, examination through a bright-field microscopy (BF) or also known as the Compound Light Microscope. microscope was carried out with four treatments (Bio-Arc, Bion, Frankincense oil and Cyanobacteria) with untreated control (treated with water only) and check control treated by the fungicide pilizol (25% propiconazole EC 200 cm^3 100L water$^-$). Fresh uredospores were sprayed on sterilized cleaned glass slides in an *in vitro* setting **(Nair and Ellingboe, 1962).** After adding various treatments, a thin layer of 2% water agar was applied to the slides. Slides were positioned in sterile Petri-dishes with wet filter papers on V-shaped glass rods. To calculate the percentage of spore germination, slides were examined under a microscope at an × 40 magnification after being incubated at 25 °C for 12 hours under full light **(Reifschneider *et al.*, 1985).** According to **Menzies and Belanger (1996),** the formation of a germ tube indicated that the spores germinated, regardless of its width. On a slide, percentages of germination for one hundred spores were computed. For every treatment, three slides were inspected.

Scanning electron microscopy (SEM)

To examine the morphology of shape and amount of spores in pustules a scanning electron microscope (SEM) was utilized. Investigations were conducted using the four tested treatments with untreated control and check control to study their ability to inhibit the uredospore's germination. Before and after infection by 24 hours the wheat seedlings were sprayed with the four tested treatment solutions, where wheat grains planted in plastic pots filled with sterilized soil and after seven days (the seedling stage), they were inoculated with uredospores suspension of leaf rust *P. triticina*. The pots were left in the dark at 20 °C for 24 h in dew chamber (100% relative humidity) then the inoculated pots were transferred into the other part in the greenhouse where there is suitable light for growth and sporulation of *P. triticina*, where the temperature was 20 ± 2°C with approximately 80% relative humidity until the appearance of disease symptoms. Afterward, the second leaf pieces were collected from each treatment (roughly 1 cm2) and put into a relaxant solution. (glutaraldehyde in sodium cacodylate buffer, 0.1M, pH 7.2). The samples were subsequently dried for 10 minutes each using a graded ethanol series (30–100%). A sputter coater (FDU-010) was used to coat the samples with gold after they had been dried using a critical point dryer (TEC-030). JEOL 100 CX-II ASID-4D, Japan, **(Caldwell and Iyer-Pascuzzi, 2019)**. A scanning electron microscope, was used to examine the samples.

Laboratory studies

Enzymes activity

The activity of oxidative-reductive enzymes, *e.g.* polyphenol oxidase (PPO), peroxidase (PO), and catalase (CAT) was estimated at seven days after inoculation, to the tested treatments. The measurements were carried out using wheat treated leaves. After homogenizing fresh leaves (0.5 g), they were centrifuged at 4 °C for 20 minutes at 12,000 rpm. Spectrophotometry was used to estimate the total soluble enzyme activity in the supernatant. PPO activity was assessed using **Malik and Singh (1980)** method. We measured POX activity using the method recommended by **Hammerschmidt *et al.* (1982).** While, catalase (CAT) activity was estimated using **Aebi (1984)** method.

GC-MS analysis

The method of phytochemical analysis of methanolic extracts of wheat leaves, at seedling stage, seven days after the appearance of disease symptoms, was used to identify organic compounds and their area sum percentage in cell the tested treatments. For eight hours, leaves were dried in an oven with recirculating air at 45˚C. In a knife mill, dried leaves were ground into a powder with a particle diameter of 2 mm. By macerating sixty-seven grams of the drug was dried and pure methanol was extracted over the course of eight days in a closed container in total darkness. Activated silica gel (60–200 mesh) was used in a chromatography column to fractionate the residue (7 g), after the extract was dried in a rotary evaporator. As solvent systems, hexane, three different ratios of hexane to ethyl acetate were used: 90:10, 80:20, and ethyl acetate. Column chromatography was then used to separate the samples once more, and a Gas Chromatography-Mass Spectrometry coupled system (GC-MS) was used to analyze the fractions and determine the outcomes **(Patricia *et al.*, 2013**). GC-MS analysis was estimated in the Regional Center for Food and Feed, Agric. Res.Cent., Egypt.

Greenhouse experiments

Four tested treatments; Bio-Arc, Bion, Frankincense oil, Cyanobacteria with untreated control and check control were tested in a greenhouse during the seedling and adult stages to wheat leaf rust caused by *P.triticina* at Wheat Dis. Res. Dept., Plant Pathol. Res. Inst., ARC, Giza, Egypt during 2023/24 growing season.

For preparation the seedling stage, the seeds of Gemmeiza11 cultivar (5-7 seeds) were sown in 10 cm clay-filled plastic pots (400 g). Seven days after sowing, the uredospores of leaf rust race (NKRGS), which were kindly provided by Wheat Dis. Res. Dept. Plant Pathol. Res. Inst., ARC, Giza, Egypt were used to inoculate the plants. Talcum powder and spores were combined at a ratio of one to twenty (v/v) according to **Tervet and Cassell, (1951)**. The seedling responds differently depending on the type of infection it received, marking it as susceptible (S) or resistant (R). According to the **Stakman *et al.* (1962)** scale, infection types 0, 0 ; 1, and 2 were regarded as resistant (R) or low infection types (L), whereas 3 and 4 were regarded as susceptible (S) or high infection types (H) as displayed in (Table 1). The tested treatments, were tested to wheat leaf rust before and after inoculation of plants by 24 hours, compared to the untreated control.

Table (1). Wheat leaf rust infection types used in disease assessment for seedling stage according to Roelfs *et al.*, (1992).

Host	response (class)	Infection type	Disease symptoms
Resistant	Immune	Low 0	No uredia or other macroscopic sign of infection
	Nearly immune	Low 0;	No, uredia but hypersensitive necrotic or chlorotic flecks present
	Very resistant	Low 1	Small uredia surrounded by necrosis
	Moderately resistant	Low 2	Small to medium uredia surrounded by chlorosis or necrosis
Susceptible	Moderately susceptible	High 3	Medium-sized uredia that may be associated with chlorosis
	Very susceptible	High 4	Large uredia without chlorosis or necrosis or rarely necrosis
Heterogeneous		X	Random distribution of variable sized. uredia on single leaf

Slow rusting components were estimated as incubation period (IP) and latent period (LP) was measured according to **Parlevliet (1975)**. Number of pustules cm^{2-1} was counted as described by **Parlevliet and Kuiper (1977**). Pustule size (PS) was measured with a light microscope using the formula suggested by **Broes (1989)**.

Pustule size = ¼ × π L × W

Where:

π = 3.14, L: is the length and W: is the width of each pustule.

The following equation was used to determine the effectiveness of seedling treatments:

$$\text{Efficacy \%} = \frac{\text{No: of pustules in the untreated control plants - No. of pustules in the treated plants}}{\text{No: of pustules in the untreated control plants}} \times 100$$

Twenty-five wheat grains from Gemmeiza 11 cultivar were sown in pots with a 25 cm diameter and left to reach the adult stage. Five pots (replicates) were used for each treatment , the plants were thinned into 10 per pot using the randomized complete block design (RCBD). According to **Large (1954),** artificial inoculation was performed at the booting stage (70 days old). The plants in the seedling stage were treated with the same race's spores (NKRGS), incubated for 24 hours in a dark dew chamber with 100% relative humidity, then the inoculated pots were moved to another greenhouse section, where the temperature was 20 ± 2°. Estimation the disease incidence measurements of area under the disease progress curve (AUDPC) . The final rust severity (FRS), and rate of disease increase (r-value), at adult stage. For each treatment, data were recorded from the appearance of rust symptoms until the early dough stage. These variables included final rust severity percentage, rate of leaf rust increase (r-value), and area under disease progress curves (AUDPC) **(Large, 1954)**. Rust severity was estimated by applying the adjusted Cobb's scale **(Peterson *et al.*, 1948**). Leaf rust response and disease severity of adult plants are noted in Table (2) with the

descriptions provided by **Roelfs *et al.* (1992) and Singh *et al.* (2013).**

The host response, various disease incidence , development components, area under the disease progress curve (AUDPC) , the final rust severity (FRS), and rate of disease increase (r-value), were used to evaluate the resistance behavior of wheat leaf rust.

The final rust severity (FRS) was recorded as outlined by **Das *et al.* (1993).**

The following formula, which **Van Der Plank (1963)** adopted, was used to estimate the rate of increase in leaf rust (r-value):

$$\text{r-value} = \frac{1}{t_2 - t_1} \left(\log_e \frac{X_2}{1 - X_2} - \log_e \frac{X_1}{1 - X_1} \right)$$

Where:

$\mathbf{X_1}$ = the percentage of the susceptible infected tissue at date t_1,

$\mathbf{X_2}$ = the percentage of the susceptible infected tissue at date t_2 and

$\mathbf{t_2 - t_1}$ = the number of days that separate these dates

Equation adopted by **Pandey *et al.*(1989)** was used to estimate the area under disease progress curves (AUDPC).

AUDPC= D [1/2 (Y1+Yk) + Y2 + Y3 + ……Yk-1]

Where:

D = number of days in between readings,

$\mathbf{Y_1}$ = first disease observation and

$\mathbf{Y_k}$ = last disease recorded.

The efficacy of adult treatments was determined using the subsequent formula:

$$\text{Efficacy \%} = \frac{\text{FRS\% in the untreated control plants - FRS\% in the treated plants}}{\text{FRS\% in the untreated control plants}} \times 100$$

Statistical analysis

Analytical statistics Using SPSS 22, a statistical analysis of variance was performed on the data. Using statistical product and service solutions (SPSS 22), of slow rusting components (latent period, incubation period, number of pustules cm^{2-1} and pustule size) and disease incidence measurements FRS and AUDPC, peroxidase (POX), catalase (CAT), and polyphenol oxidase (PPO) were carried out **(Allen *et al.*, 2014).**

RESULTS

The effects of four tested treatments; Bio-Arc, Bion, Frankincense oil and Cyanobacteria were studied using wheat plants subjected to leaf rust stress under laboratory and greenhouse conditions.

Microscopic examination

Effect of the tested treatments on spore germination

Microscopic examination was conducted to evaluate the tested therapies on disease symptoms and spore germination. The result depicts the effects of four control treatments (Bio-Arc, Bion, Frankincense oil and Cyanobacteria) with control (untreated) and check control (fungicide) on *P. triticina* spore germination on water agar medium (Fig. 1). Treatments with Bio-Arc and Bion inhibited spore germination more than the untreated control (Fig.1 A,B and E), while Frankincense oil and check control (fungicide) completely inhibited spore germination (Fig.1 C and F). Conversely, the highest germination percentage was displayed by spores treated with Cyanobacteria compared to the other three treatments mentioned previously (Fig.1 D). Microscopic analyses of uredospores

revealed the presence of anomalies, lysis, collapse, and shrinking (Fig. 1).

Table (2): Adult plant resistance response and severity for leaf rust based on the modified Cobb's scale. Peterson *et al.*,(1948) and the reaction types by Roelfs *et al.*, (1992) and Singh *et al.*, (2013).

Disease response	Disease severity %	Host response	Symptoms
R	0-5	Resistant	Resistant no visible infection or some chlorosis or necrosis and no uredia
R-MR	10-20	Resistant to Moderately Resistant	
MR	20-30	Moderately Resistant	Moderately Resistant small uredia present ant surrounded by either Chlorotic or necrotic areas
MR-MS	30-40	Moderately Resistant to Moderately Susceptible	
MS	40-50	Moderately Susceptible	Moderately Susceptible medium-sized uredia present and possibly surrounded by chlorotic areas
MS-S	50-70	Moderately Susceptible to Susceptible	
S	70-100	Susceptible	Susceptible large uredia present, generally with little or no chlorosis and no necrosis

R = Resistant; M = Moderately; S = Susceptible

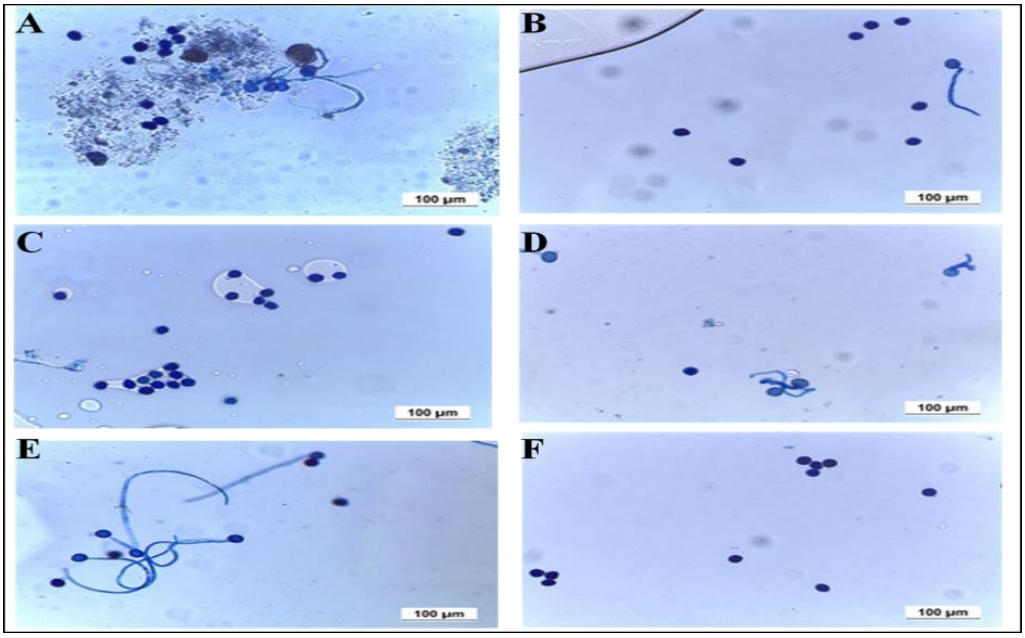

Fig. 1 Light microscopy of *P. triticina* uredospores germination on water agar medium with four treatments, Bioarce A, Bion B, Frankincense oil C, Cyanobacteria D with Control (untreated) E and Check control (fungicide) F.

Scanning electron microscope (SEM) examination

The shape and amount of spores in pustules were examined using a scanning electron microscope (SEM). In order to validate the light microscopy results for spore germination, bio treatments; Bio-Arc, Bion and Frankincense oil were applied to the wheat leaves in comparison to the control. These treatments inhibited spore germination and decreased the amount of spores in pustules with non-germinated uredospores in the pustules and more shrunken spores in them. (Fig.2 A,B and C). The Cyanobacteria (Fig.2 D) displayed semi normal pustules shape and amount of spores like untreated control (Fig.2 E).

Laboratory studies: -

Activity of oxidative enzymes

At seven days after inoculation, the activities of the oxidative-reductive enzymes peroxidase (POX), polyphenol oxidase (PPO) and catalase (CAT) were stated (Fig.3 A,B and C). In this regard, Bio-Arc showed the highest values of PO, PPO, and CAT, being 0.048, 1.407, and 1.003, respectively, followed by Frankincense, being 0.045, 0.709 & 0.866 oil and Cyanobacteria , being 0.041, 0.618 & 0, respectively, compared with untreated control plants. While treatments, Bion and check control (fungicide) showed the lowest levels of enzymes activity (Fig. 3 A,B and C).

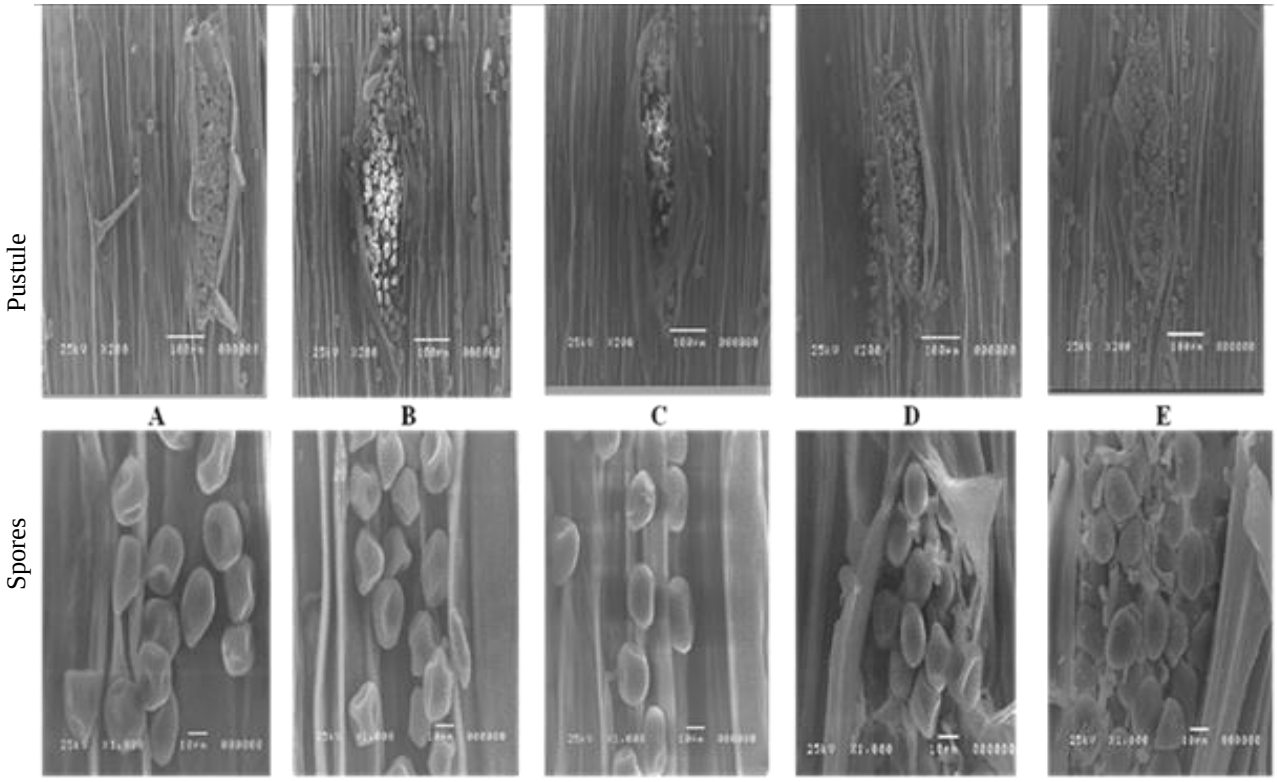

Fig. 2 SEM micrographs showing the effect of the four tested treatments on pustuls and uredospores of *P. triticina* , Bioarce A, Bion B, Frankincense oil C, Cyanobacteria D with Control (untreated) E.

GC-MS analysis

Using a GC-MS system, several compounds at different rotation times/min (Fig.4) were identified in the extract of wheat leaves treated with four different bio and both Control (untreated) and check control (fungicide) from fungal infection during the seedling stage under the greenhouse conditions (Table 3). The highest percentage of compounds in the tested treatments were , Citronellol, Linolenic acid, Pratensein, 5β,7βH,10α-Eudesm-11-en-1α-ol, 15-Tetracosenoic acid, Geranyl isovalerate, (-)-Gallocatechin, Linoleic acid, Phytanic acid, Eicosen-1-ol, cis-9-, Hexa-hydro-farnesol, Phytol, Docosanoic acid, 7,4'-Homoisoflavane, Casticin, Nonacosane, and β-Sitosterol that ranged from 1 to 15.14% area sum /cell (Fig. 5), The remaining ingredients were present in trace amounts, the four compounds (Linolenic acid, 5β,7βH,10α-Eudesm-11-en-1α-ol, Pratensein and Casticin) had the highest values of area sum % at bio treatments Bioarce, Bion and Frankincense oil which considered of moderately and highly efficacy % treatments against wheat leaf rust infection compared to untreated control. On the other hand, compounds, e.g. Hexa-hydro-farnesol, 15-Tetracosenoic acid, Phytol, Geranyl isovalerate, and Eicosen-1-ol, cis-9, were found to have high values in treatments with low efficacy; these compounds may be related by pathogenicity between the cyanobacteria and the untreated control.

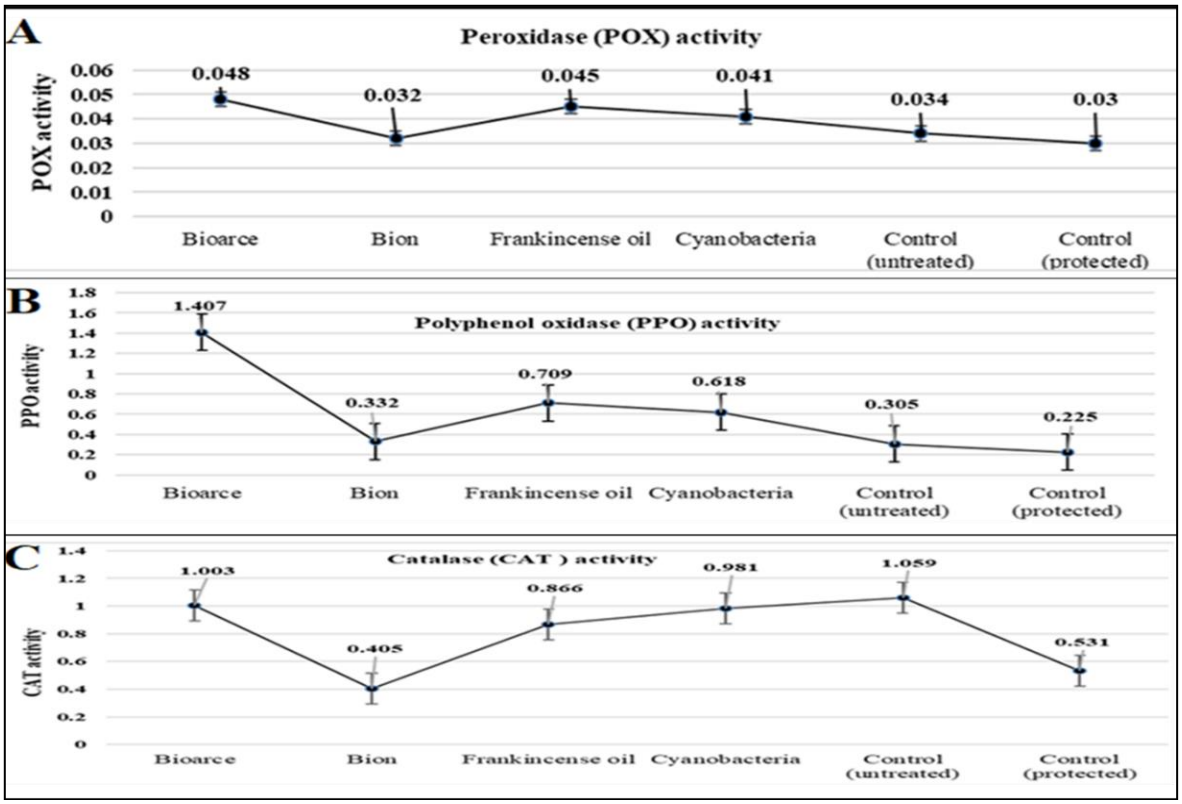

Fig.3 Activity of peroxidase (POX) A, polyphenol oxidase (PPO) B, and catalase (CAT) C enzymes in wheat leaves infected with *Puccinia triticina* and treated with four treatments ; Bioarce, Bion, frankincense oil, Cyanobacteria with Control (untreated) and Check control (fungicide)
L.S.D at 0.01% for (POX), (PPO) and (CAT) are 1.520, 0.387 and non significant respectively.

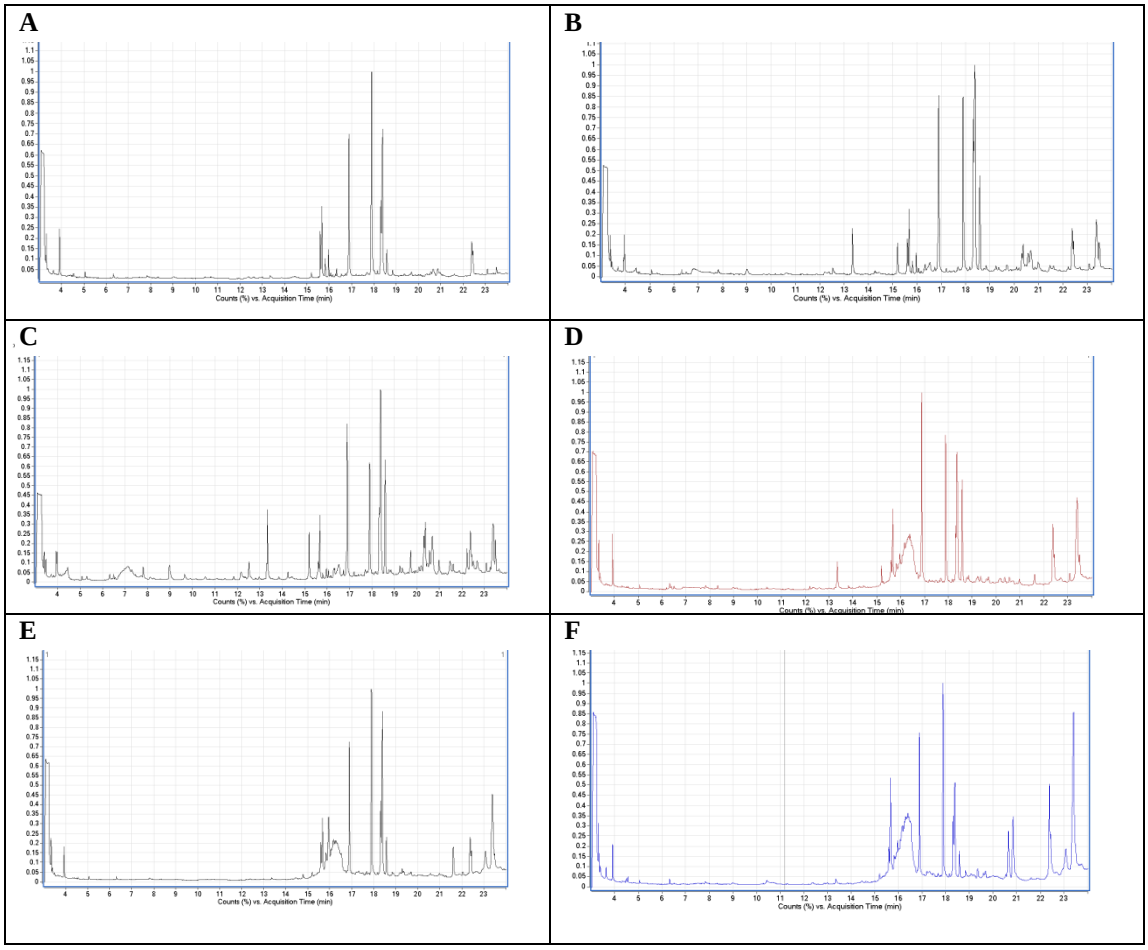

Fig. 4 GC-MS chromatograms rotation time /min of several organic compounds (listed in Table 3) at four treatmens , Bioarce A, Bion B, Frankincense oil C, Cyanobacteria D with Control (untreated) E and Check control (fungicide)F found in the leaves of wheat plants infected by leaf rust , previously treated by the tested treatments, grown at the seedling stage under greenhous conditions.

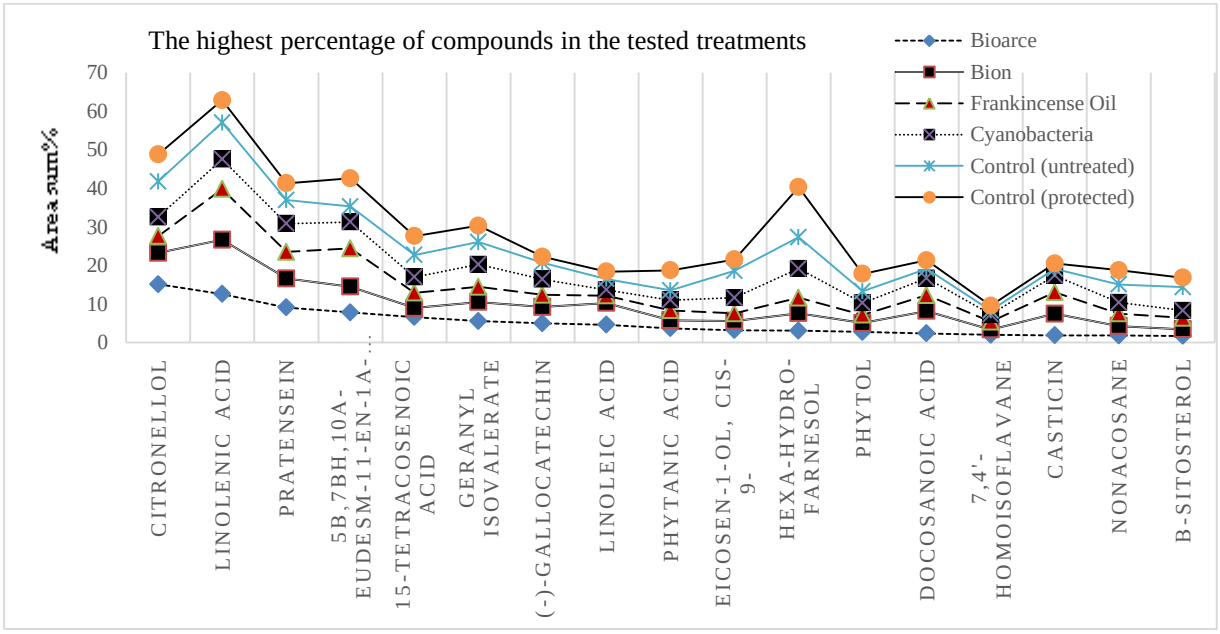

Fig.5 The highest percentage of compounds found in wheat leaves treated with the tested treatments, Bioarce, Bion, frankincense oil, Cyanobacteria with Control (untreated) and Check control (fungicide).

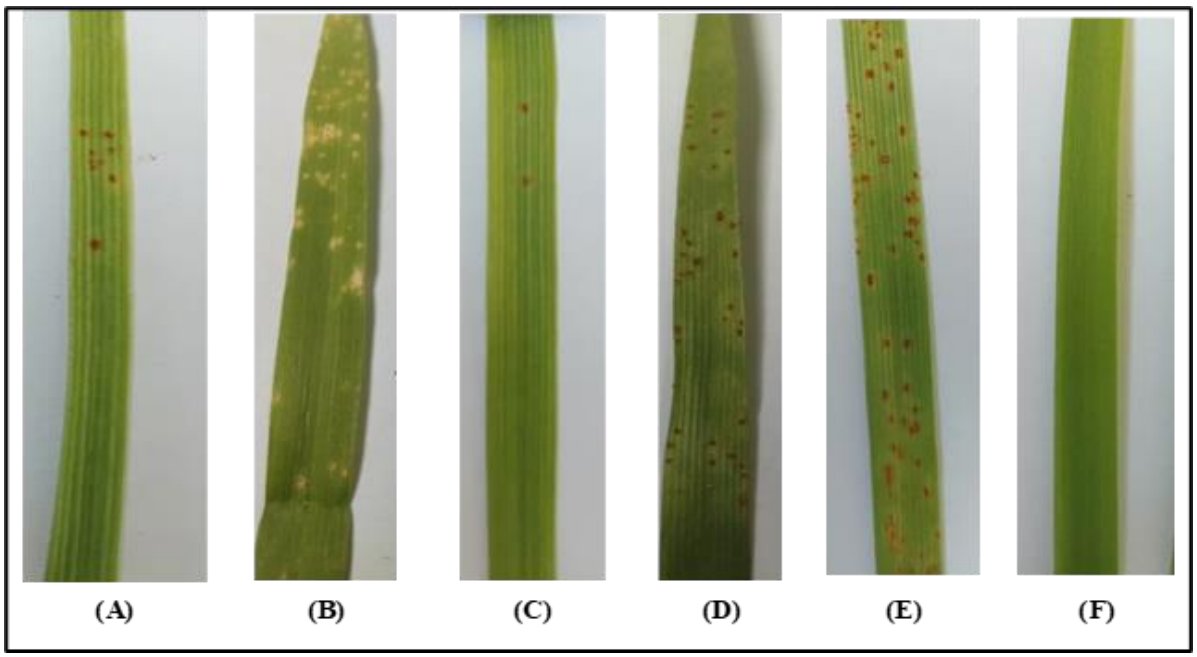

Fig. 6 Effect of four treatmens Bioarce A, Bion B, Frankincense oil C, Cyanobacteria D with Control (untreated) E and Check control (fungicide) F on wheat leaf rust reaction at seedling stage during growing season 2023/24 under greenhouse condition.

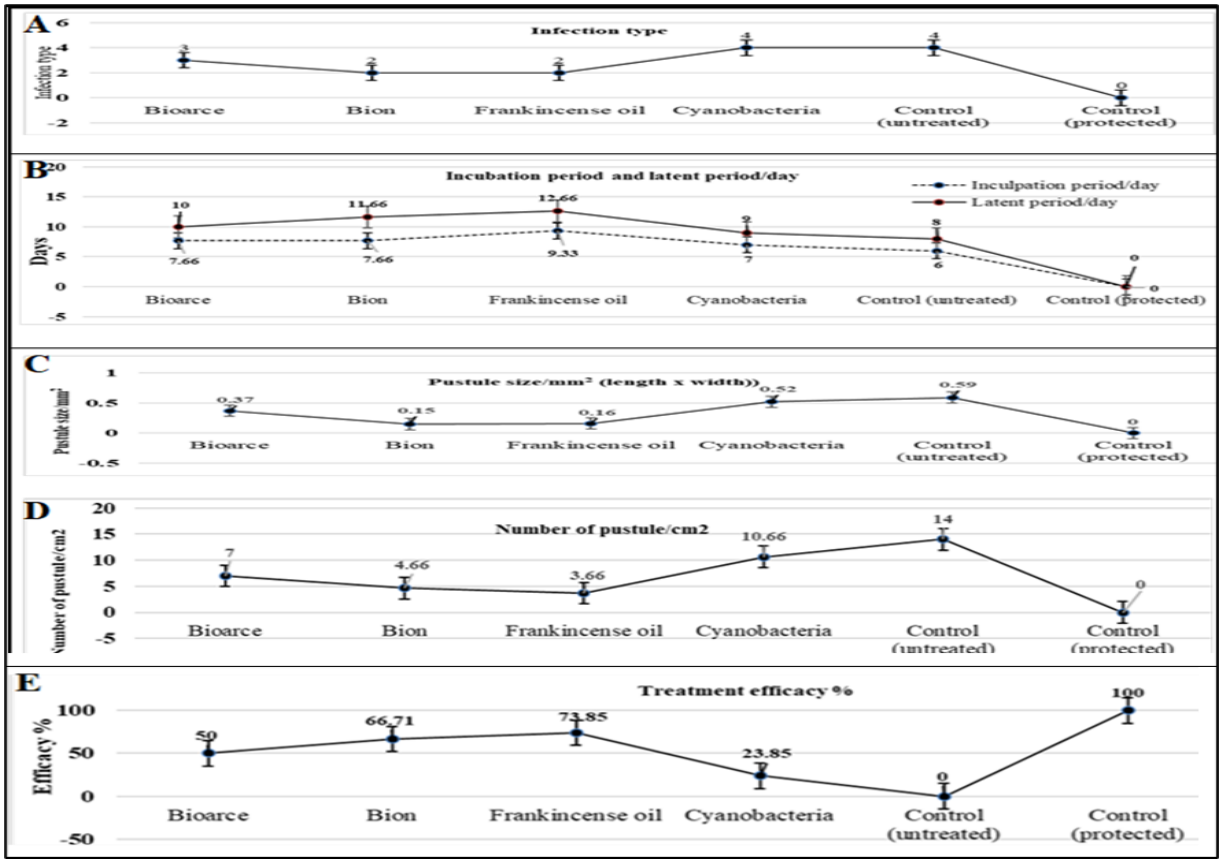

Fig. 7. Effect of four treatments; Bioarce, Bion, Frankincense oil, and Cyanobacteria on infection type (A) , incubation period, latent period (B), Pustule size (C), number of pustule/cm^2(D) and their efficacy (E) on wheat leaf rust disease at seedling stage during 2023/24 under greenhouse condition.

L.S.D at 0.05% for Incubation period/day, Latent period/day (B) ,Pustule size/mm2 (length x width)(c) and numbero of pustule/cm^2 (D) are 1.16, 0.722, 0.23 and 3.54 respectivey.

Table 3. Compounds found in wheat leaves treated with four different treatments Bioarce, Bion, Frankincense oil, Cyanobacteria with Control (untreated) and Check control (fungicide) from fungal infection during the seedling stage under the greenhouse condition.

NO.	Rotation time (min)	Compound	Area Sum %					
			Bioarce	Bion	Frankincense oil	Cyanobacteria	Control (untreated)	Control (protected)
1	3.37	p-Menthan-8-ol	0.63	0.66	0.49	1.01	0.39	1.09
2	3.908	7,4'-Homoisoflavane	2	1.37	2.06	1.9	1	1.24
3	4.338	2-Undecanol	0.72	0.92	2.51	1.08	0.48	1.08
4	4.756	Myricetin	0.41	0.45	0.65	0.55	0.22	0.85
5	5.052	Farnesol	0.45	0.66	0.58	0.66	0.23	1.03
6	5.302	4'-Hydroxy-2'-methyl-3,4,5-trimethoxychalcone	0.34	0.22	0.75	0.6	0.24	0.62
7	5.63	Nopol	0.21	0.34	0.27	0.44	0.11	0.42
8	6.323	Asarone	0.47	0.53	0.81	1.24	0.42	0.82
9	6.503	Borneol	0.33	0.47	0.92	0.75	0.26	0.58
10	7.808	Dodecanal	0.23	0.44	0.99	0.69	0.25	0.43
11	8.328	Gentisic acid	0.15	0.36	0.26	0.41	0.22	0.5
12	8.997	Caryophyllene	0.85	1.1	1.84	0.96	0.28	1.04
13	10.428	Carvacrol	0.34	0.31	0.45	0.77	0.13	0.87
14	10.678	p-Cymen-7-ol	0.61	0.8	1.07	1.02	0.22	0.82
15	11.314	Erucic acid	0.42	0.54	0.61	0.82	0.12	0.64
16	11.847	4',5,7-Trihydroxy 3,6,8-trimethoxyflavone	0.2	0.18	0.55	0.46	0.26	0.52
17	12.343	4-Hydroxy-β-ionone	0.22	0.73	1.01	0.53	0.34	0.8
18	12.536	cis-9-Hexadecenoic acid	0.47	0.74	1.28	0.68	0.57	1.13
19	13.335	Resveratrol	0.25	2.07	2.72	1.24	0.21	0.78
20	13.844	5,7-Dihydroxy-3',4',5'-trimethoxyflavanone	0.24	0.41	0.9	0.58	0.12	1.19
21	14.418	(-)-Catechin gallate	0.44	0.72	0.98	2.15	0.96	1.73
22	15.197	Isoquercitin	0.29	1.04	1.53	0.41	1.02	0.2
23	15.587	3',4',5',5,6,7-Hexamethoxyflavone	1.44	1.25	0.73	3.21	4.27	0.83
24	15.653	Geranyl isovalerate	5.58	4.92	3.96	5.81	5.82	4.24
25	15.968	Eicosen-1-ol, cis-9-	3.19	2.42	1.97	4.04	6.98	2.97
26	16.333	Rhamnazin	0.58	1.24	1.16	2.09	2.82	1.46
27	16.514	Nonacosane	1.82	2.47	3.24	2.86	4.68	3.7
28	16.878	Pratensein	9.11	7.52	6.87	7.37	6.09	4.37
29	17.887	Citronellol	15.14	8.13	4.33	4.95	9.22	7.01
30	18.306	Linoleic acid	4.63	5.65	1.88	1.52	2.81	1.91
31	18.372	Linolenic acid	12.6	14.05	13.18	7.69	9.52	5.82
32	18.576	Casticin	1.9	5.57	5.56	4.38	1.73	1.39
33	19.618	15-Tetracosenoic acid	6.63	2.33	3.98	4.03	5.75	4.9
34	20.315	5β,7βH,10α-Eudesm-11-en-1α-ol	7.8	6.68	9.96	6.81	4.06	7.28
35	20.832	Lutein	0.68	1.95	1.07	2.66	1.72	6.82
36	21.595	(-)-Gallocatechin	4.98	4.26	3.13	4.04	4.32	1.55
37	22.374	Phytol	2.77	2.32	2.1	3.07	3.05	4.52
38	22.431	Phytanic acid	3.69	2.08	2.59	2.67	2.52	5.19
39	23	β-Sitosterol	1.72	1.75	2.94	1.92	6.06	2.43
40	23.391	Hexa-hydro-farnesol	3.08	4.54	4.03	7.55	8.11	13.01
41	23.477	Docosanoic acid	2.4	5.81	4.11	4.39	2.43	2.21

Greenhouse studies

The effects of the four tested treatments *i.e.*, Bio.Arc (A), Bion (B), Frankincense oil (C), Cyanobacteria (D) compared with control (untreated) (E), and check control (fungicide) on the disease symptoms of wheat leaf rust (F) are presented in Fig. (6). As shown in Fig. (7),these treatments, also, display slow rusting components estimated as an infection type (A), incubation period, latent period (B), pustule size (C), number of pustule cm^{2-1} (D), and their efficacy (E) to development wheat leaf rust at seedling stage. The values of incubation period, latent period and efficacy% were low in plants treated with Bio-Arc and Cyanobacteria (7.66 & 7.00 day), (10.00 & 9.00 day) and (50 & 23.85%), respectively (Fig.7 B&E), and had high values of infection types, pustule size mm^2 and number of pustule cm^{2-1} were (3 & 4), (0.37 & 0.52 mm^2) and (7.00 & 10.66 cm^{2-1}), respectively (Fig.7 A,C and D),. While the rest treatments, Bion and Frankincense oil showed high efficacy% of incubation period, latent period (7.66 & 9.33 day), (11.66 & 12.66 day) and (66.71 & 73.85%), respectively, and also had the lowest values of infection types, pustule size mm^2 and number of pustule cm^{2-1} . The corresponding values were (2&2), (0.15 & 0.16 mm^2) and (4.66 &3.66 cm^{2-1}), respectively, compared to untreated control at seedling stage (Fig. 7 A,B,C,D, & E). When bio treatments, particularly Bio.Arc, Bion and Frankincense oil were sprayed on foliar of wheat seedlings, the infection type, pustule size, and number of pustules cm^{2-1} were decreased in comparison to the untreated control. This resulted in the highest resistance response of wheat plants against the leaf rust pathogen, with 66.71 and 73.8 % efficacy, respectively. While Bio-Arc treatments had a 50% efficacy when compared to the untreated control.

Data of the adult plant stage (Fig. 8 A, B, C, and D) demonstrated the impact of the four treatments on the incidence assessment of wheat leaf rust e.g., final rust severity% (FRS) (A) , rate of disease increase (r-value) (B), area under disease progress curve (AUDPC) (C) and their efficacy (D). The two bioagents treatments i.e., Bion and Frankincense oil had low values of disease incidence assessment i.*e.*,FRS %, r-value and AUDPC .The corresponding values were (9.13 & 6.66), (0.007 & 0.003) and (100.41 & 128.23), respectively and had high efficacy (84.78 and 88. 9%), respectively. While, Bio-Arc and Cyanobacteria had high values of disease incidence assessment *i.e.*,FRS %, r-value and AUDPC , which recorded (31.66 & 46.66), (0.253 & 0.366) and (315.92 & 942.46), respectively with moderately efficacy, being 47.23 % for Bio.Arc treatment, while Cyanobacteria had low value of efficacy, being % 22.23. In general, the foliar spray application of wheat plants at the adult stage with any of the tested control agents has significantly reduced the incidence of leaf rust infection *i.e.*,final rust severity%, rate of disease increase, and area under disease progress curve(Fig.9 A, B,C,D,E and F).. However, treatments with an efficacy of up to 88.9%, Bion and Frankincense oil, were the most successful treatments.

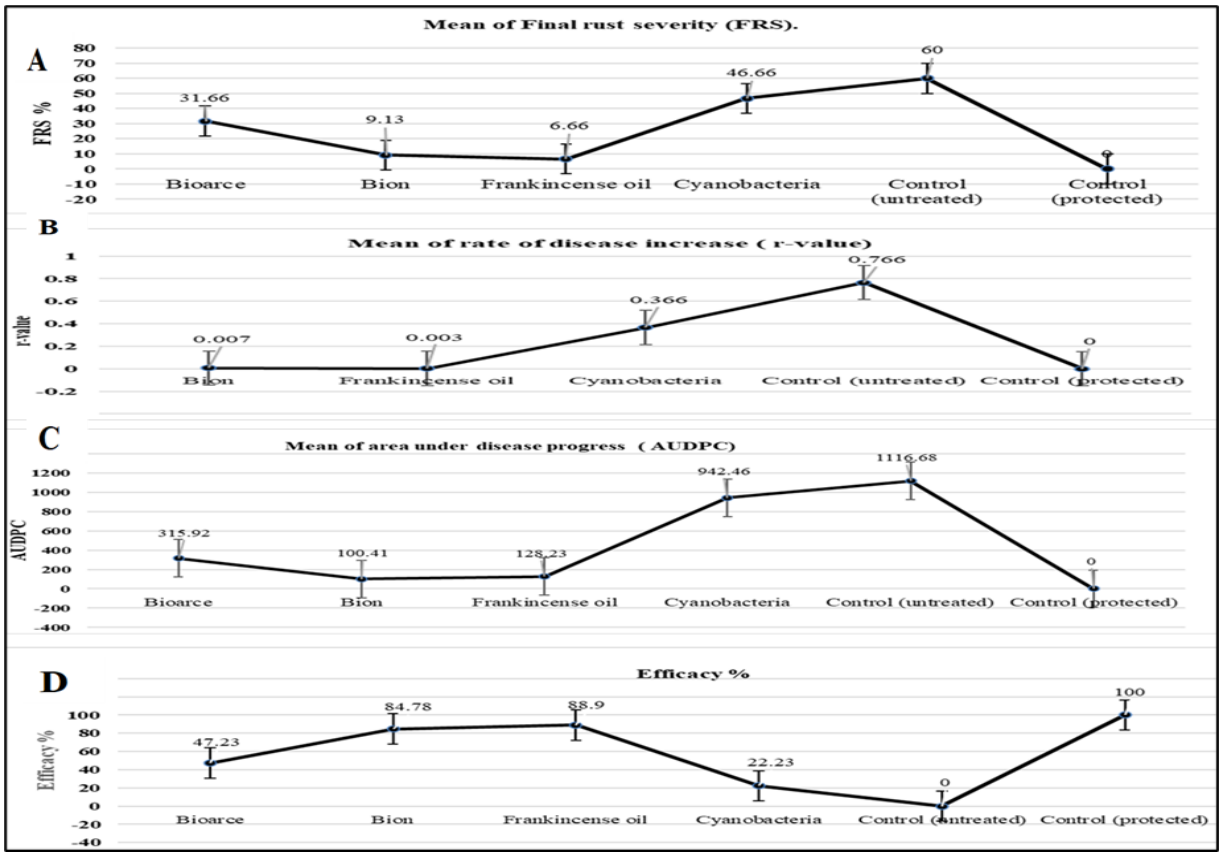

Fig. 8 . Effect of four treatments ; Bioarce, Bion, Frankincense oil, and Cyanobacteria on mean of final rust Severity (FRS) (A) , mean of rate of disease increase (r-value) (B), Mean of area under disease progress (AUDPC) (C) and their efficacy (D) on wheat leaf rust disease at adult stage during growing season 2023/24 under greenhous condition.

L.S.D at 5% to Final rust severity (A), Rate of disease increase (B) and Area under disease progress curve (C) are 17.078, 0.140 and 34.42 respectivey.

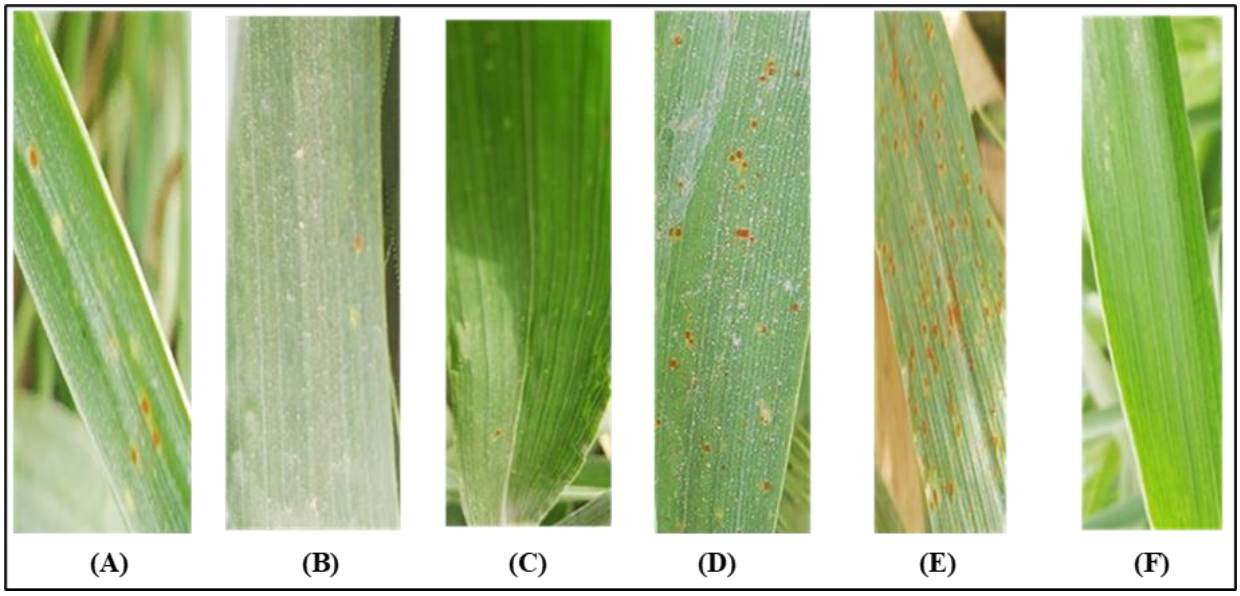

Fig. 9 Effect of four treatmens Bioarce A, Bion B, Frankincense oil C, Cyanobacteria D with Control (untreated) E and Check control (fungicide) F on wheat leaf rust reaction at adult stage during growing season 2023/24 under greenhous conditions.

DISCUSSION

Data presented herein showed that growth of *P. triticina* was markedly inhibited in plants treated with Bion and Frankincense oil. Thus, it is likely that the well-known priming agents of Bion and Frankincense oil triggered a quick and strong reaction in the host plants in response to fungal invasion (**Zimmerli *et al.*, 2000; Ton and Mauch-Mani, 2004 and Hulten *et al.*, 2006**), as well as Frankincense oil, were capable of suppressing *P. triticina's* mycelium growth and spore germination. This supported that these oils could serve as a substitute for artificial fungicides in the control of such pathogenic fungus as mentioned by (**Julara*t et al.*, 2009**). Similar findings have been reported, suggesting that plant resistance inducers may have a toxic effect on the growth of pathogens. According to **Porat *et al.* (2003) and Shabana *et al.* (2017)** inducers of plant resistance offer a viable substitute for managing fungal diseases **(Wang and Zhou, 2018** and **Chaudhary and Shukla, 2019)** and the potential to lessen the severity of *P. triticina* the causal of wheat leaf rust.

The observations of several parts in an infected and untreated wheat leaf by a scanning electron microscope revealed disorganized cells with altered ultrastructure and thick cell walls surrounding them. Numerous degenerate chloroplasts and electron-dense particles were present in the cells. The treatments that can be utilized as biocontrol agents (BCAs) were identified during the current study as being Bio-Arc, Bion and Frankincense oil. The study, also, identified potential pathways that, when combined with the entophytic effect of the tested bio treatments, which stimulate wheat's defense system to suppress leaf rust spores. This either eliminates the plant pathogen, slows down its growth indirectly boosts host cell immunity or inside the host, by reducing cell damage and pathogen progression through an ISR (induced systemic resistance) mechanism (**Martínez-Hidalgo *et al.*, 2015).** This mechanism is crucial for promoting plant growth and activating a plant defense mechanism by causing plants to develop induced systemic resistance (ISR) (**Compant *et al.*, 2005)**. The plants produce a variety of active compounds with antimicrobial and antifungal qualities that can aid the plant in fending off invaders and may lessen the severity of the disease by preventing the growth of uredospore's, which include ruptured spores and non-germinating spores with germ tubes smaller than spore radius. In certain instances, it was also discovered that the germ tubes had been harmed by malformed morphology, such as shrinkage or swelling. SEM analysis of the treated leaf samples corroborated these findings. Therapy, decreased illness severity, and altered type of response were noted and the bio treatment tested cells' competition with the fungal spores for the site of entry and metabolite release, which prevented the elongation of germ tubes, was linked to a decrease in the severity of the disease and, also, demonstrated elevated Reactive Oxygen Species (ROS) generation through the assessment of antioxidant enzyme

activity and pathogenicity expression **(Sessitsch *et al.*, 2012).**

Antioxidant defense-related enzymes demonstrated the greatest increase by encouraging plant resistance to the pathogenic agents. These enzymes may take a part in the responsive defense mechanism **(Ray *et al.*, 1998).** Similarly, using Bio-Arc, Frankincense oil and Cyanobacteria , increased CAT, POX, and PPO enzymatic activity. One of the oxidative enzymes, peroxidase (POX) enzyme is crucial for boosting host resistance to control plant diseases **(Liau and Lin, 2008)**. It is closely linked to the enhanced capacity of systemically protected tissues for lignification and is recognized to catalyze the last polymerization stage of lignin formation **(Chittoor *et al.*, 1999).** According to **Hameed *et al.* (2011)**, the peroxidase activity may therefore be a useful marker of a plant's ability to disease resistance. Additionally, polyphenol oxidase (PPO) contributes significantly to plant defense by oxidizing endogenous phenolic compounds to the deadly pathogens that invade plants with quinine **(Thakker *et al.*, 2011).** According **to Hanifei *et al.* (2013),** it might, also, take a part in the inducible defense reaction and hypersensitivity that cause plants to become resistant to bacteria, viruses, and fungi. It is commonly known that phenolics, which are toxic compounds that control a plant's antioxidant capacity, play a significant role in the immune system of plants. As a result, their induction is regarded as a sign of plant resistance. Our research revealed an increase in the total phenols content and defense-related enzymes in wheat plant's, which suggests that they have a stimulating effect on the plant's defense mechanism against the wheat leaf rust disease. The results obtained by **Da Silva *et al.* (2017)** against *Scytalidium lignicola* on cassava plants are consistent with these findings. Regarding this, **El-Sharkawya *et al.* (2018)** looked into the control of stem rust disease in wheat using various combinations of *Carbuncular mycorrhizal* and *Trichoderma* spp. These combinations significantly decreased disease measures, induced the polyphenol oxidase and peroxidase enzymes, raised the amount of phenol overall, and enhanced the examined growth and yield characteristics. In order to activate systemic acquired resistance and regulate the expression of defense genes, a secondary signal is sent. The elevated activity of catalase inhibited the rise in cytosolic hydrogen peroxide, which produces toxic circumstances and stops the spread of pathogens **(Neamat *et al.*, 2016).** According to **Milavec *et al.* (2001),** peroxidase is the first enzyme to show alterations in its activity when exposed to environmental stress. Furthermore, it is well established that modifications to this antioxidant enzyme directly contribute to the initiation of plant defense mechanisms. Antioxidant up regulation shields plant cells against ROS-induced oxidative bursts. This explains why antioxidant-scavenging enzymes were up-regulated in resistant cultivars **(Hafez *et al.*, 2012 and 2014).**

Using a GC-MS system, forty one organic compounds were identified, these organic substances are activating, stimulating, or boosting plant defense mechanisms without coming into contact with infection directly. Certain beneficial bacteria work with plants to promote host immunity or develop host resistance **(Javaid, 2006 and Conrath *et al.*, 2015).** These agents comprise natural products and chemical compounds derived from various sources, including gene products, synthetic chemicals, microbial metabolites, and plant extracts **(Pal and Gardener, 2006).** It is evident that some of these inhibitory compounds, perhaps as a result of increased hormone production, which improve plant vigor and suppress plant diseases **(Dun-Chun *et al.*, 2021).** Essential oils are known to interact with the membranes of cells, which may have an effect on different components of the membrane, such as transport systems, enzymes, receptors, and ion channels. Literature has confirmed the synergistic effects of sesquiterpenes (like β-caryophyllene) and monoterpenes (like pinene). In other study, the chemical compounds myrcene and α-humulene found in the essential oil under study demonstrated significant antibacterial activity against both Gram-positive and Gram-negative bacteria as well as some yeasts. As a result, the essential oil used in this study contained substances that have been shown to have antimicrobial effects on molds, yeasts, and bacteria, which may explain its broad antimicrobial activity **(Badria, 2015; Ali and Bożena, 2016 and Al-Kharousi *et al.*, 2023).** Fatty acids, such as linolenic acid, are biostimulants **(Moghith, 2019 and Amer *et al.*, 2024)** and these may be the reason behind the efficacy of Bio-Arc, Bion, and Frankincense oil as these treatments have more than 12% of biostimulants. Group of organic acids Docosanoic acid, Phytanic acid, Gentisic acid, 15-Tetracosenoic acid, and cis-9-Hexadecenoic acid were found to have high value at treatments Frankincense oil, Bio-Arc, and Bion that prevent wheat leaf rust infection development and could be the responsible of treatments efficacy. Similar results were decided by **Nawrocka, and Małolepsza, (2013) and El-mohamedy (2017)**.

When treatments, particularly Bio-Arc, Bion and Frankincense oil were sprayed on foliar of wheat seedlings, the infection type, pustule size, and number of pustules cm^{2-1} were decreased in comparison to the untreated control. The same results were decided by **Omara *et al.*(2020),** who found that when bio treatments were applied, values of the infection type, pustule size, and number of pustules were all lower than untreated Bio-Arc treatment had a 50% efficacy when compared to the untreated control. Bio-Arc 6% is commercial bio product with active ingredients *Bacillus megaterium*. Bacilli, also, act antagonistically by secreting extracellular metabolites, including siderophores, antibiotics, and cell wall hydrolases. *Bacillus* species, also, encourage systemic resistance, which enhance the plant's ability to with stand pathogen invasion. *Bacillus* species, also, engage in actions that promote plant growth, such as solubilization, phosphate nitrogen fixation, and

phytohormone production. The use of antagonistic and plant growth-promoting Bacillus strains could be beneficial in the development of commercially viable treatments **(Miljakovic *et al*., 2020 and El-Sayed *et al*., 2022**). Moreover, Bio-Arc can be producing at least 66 distinct antibiotic compounds, which makes them antagonistic to plant pathogenic fungi **(Ferreira *et al*., 1991)** Eugenol is an active component that has been effectively utilized to eradicate numerous plant pathogens by preventing *P. striiformis* uredospores from germination and Eugenol treatment reduced latent and incubation periods during the seedling stage AUDPC and rust severity (%) at the adult stage **(El-Sawy *et al*., 2016).**

Conversely, Cyanobacteria efficacy on wheat leaf rust was low effective. In contrast, Cyanobacteria treatment improved vegetative growth and plant biomass and increased chlorophyll pigment, which led to the increase in the disease cycle and the number of generations and symptoms because Cyanobacteria fix N_2 in the atmosphere to be useful for plants *Cycad, Gunnera,* and so on. Some members of Cyanobacteria are equipped with specialized cells (heterocyst's with modified thick-walled cells), which are considered nitrogen-binding sites by nitrogenize enzyme is a complex that catalyzes the conversion of N_2 molecules into reduced forms, such as ammonia **(Singh *et al*., 2011**). Following cell death, secretion or microbial degradation can release fixed nitrogen in the form of peptides, ammonia, free amino acids, vitamins, and substances resembling auxin (**Subramanian and Sundaram, 1986).**

CONCLUSION

Data of pot experiment conducted under greenhouse conditions on seedling or adult stages revealed that all tested treatments, including Bio-Arc, Bion, Frankincense oil and Cyanobacteria , could, to varying degrees, reduce the growth of the disease response caused by wheat leaf rust, where their effectiveness increased to 88.9% at the adult stage and 73.85% at the seedling stage. This shows the ability of these treatments in controlling leaf rust disease of wheat. So in managing plant diseases, biological control is thought to be a promising substitute for fungicides and plant resistance; however, its endorsement will require a deeper comprehension of the ways in which its natural and societal functions interact. The interaction between environments and plant pathogens is changed by the introduction of bio-control agents (BCAs), which result in physical and biological cascades that impact plant health, ecological function, and pathogen fitness. As a result, the philosophy of disease control should change from being a monolithic concept that solely addresses crop productivity to one that addresses crop productivity as well as affordable accessibility, social acceptability, and ecological function in order to attain these goals. Efforts should be made the creation of "green" BCAs and their synthetic and dynamic integration with other disease control

strategies in an integrated disease management plan.

Author's contribution

Majority contribution for the whole article belongs to the author(s). The authors read and approved the final manuscript.

Competing interests

The author declares that he has no competing interests.

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