Biochemical control of wheat leaf rust by nanoparticles

Abstract

The potential antifungal activity of different applications of Biochemically synthesized silica nanoparticles (NPs), orange oil NPs, and fungicide (Crwan® 25% EC) was investigated to control leaf rust disease of wheat. The obtained data showed that disease severity significantly decreased in all treated wheat plants compared to the control. Additionally, all applications enhanced the number of grains and grain weight/spike. The significant effects of different applications increased chlorophyll, carotenoid, phenolic, and protein contents. Moreover, they increased the activities of catalase and polyphenol oxidase enzymes compared to the control. Finally, the fungicide,Crwan®, yielded the best results in our study compared to other applications, while orange oil NPs (200 ppm) were the least effective. Nanoparticles are environmentally friendly and have recently been used as a safe alternative to pesticides and chemical fertilizers in agriculture farms to reduce infectious diseases and improve crop yield.

Keywords**:** Wheat, Leaf rust, Nano-silica, orange oil NPs, Fungicide.

Introduction

Wheat (*Triticum aestivum* L.) is an important cereal crop worldwide and a staple food for about one-third of the world's population. It is essential in total cereal production and global food security (**FAO, 2020**). Leaf rust disease is caused by the fungus *Puccinia triticina*f.sp. *tritici* causes yield loss due to reduced kernel weights and decreased kernel numbers per head (**Goyeau et al., 2007; El-Orabey et al., 2020; Abd El-Rahman et al., 2021**). Leaf rust causes significant crop losses associated with geographical regions and locations (**Basnet et al. 2014a, b**).

Citrus plants belong to the Rutaceae family, which has several subtypes of plants, such as oranges, mandarins, limes, lemons, grapefruits, citrons, and bergamot plants. According to the Food and Agriculture Organization (FAO), citrus wastes contain high-quality fiber and pectin, in addition to their composition of many valuable bioactive compounds such as hesperidin, polyphenols, flavonoids, carotenoids, and essential oils (EOs). They have been used in various industrial products such as food and beverage, cosmetics, and pharmaceuticals (**Singh et al. 2020; Rafiq et al. 2018**). Hesperidin (3',5,7-trihydroxy-4'-methoxy flavanone-7-6-O-α-L-rhamnosyl-D-glucose) is a flavonoid by-product found abundantly in citrus production, mainly in sweet orange and lemon. Several reports indicated that hesperidin displays many pharmaceutical effects such as anti-allergic, antioxidant, and anti-inflammatory (**Sikdar et al. 2016**). Also, it exhibited cytotoxic activity against different rat model carcinogenesis, including the tongue, esophagus, colon, and urinary bladder (**Caristi et al. 2006**). Citrus EOs and flavonoids are widely recognized for their beneficial effects in possessing many biological activities, such as antioxidant, antimicrobial, and cytotoxic properties. They are used in food additives and cosmetics (**Ahmed et al., 2021**). These oils are extensively studied for their potential uses in the food industry. Their composition is a mixture of hydrocarbons, oxygenated compounds, and non-volatile residues, including terpenes, sesquiterpenes, aldehydes, alcohols, esters, and sterols (**Darjazi et al. 2013; Zou et al. 2016**).

Nanotechnology is an important tool of modern science that has contributed to every sector of life. Nanoparticles have a size range between 1 and 100 nm (**Bonner 1983; Nel et al. 2006**), physicochemical properties that differ from bulk materials, help to improve nutrients, and could be used as growth stimulators and as plant protection products (**Abdul-Baki and Anderson, 1973**). In recent years, nanomaterials (NMs) have been extensively investigated independently for their pharmaceutical and biological activities. Their potency is independent of size, physical properties, surface charges, stability, and others (**Wei et al. 2012**). They are employed in a wide range of applications in science and technology, such as biomedical, agriculture, electronic information technology applications, and environmental remediation (**Prasad et al., 2018**). Many studies have confirmed the ability of NMs to improve seed germination and seedling growth (**Siddiqui and Al-Whaibi 2014; Sabaghniaand Janmohammadi 2014, 2015**). Positive effects of different nanoparticles, i.e. titanium dioxide (TiO2), zinc oxide (ZnO), nickel (Ni), and chitosan (CS), on the growth of wheat seedlings were reported (**Rawat et al. 2018; Li et al. 2019**).

Silicone cation (Si) can improve plant growth and stimulate the resistance mechanisms of plants against biotic (**De Curtis et al. 2012; Filho et al. 2013**) and abiotic stress (**SabaghniaandJanmohammadi 2014, 2015**). It is known to suppress many plant diseases, such as bacterial blight, brown spot, grain discolouration, leaf scald, leaf and panicle blast, stem rot, and sheath blight in rice, as well as powdery mildew in wheat and cucumber (**Rodrigues et al. 2015**). In wheat, (Si) significantly reduced many fungal diseases, such as powdery mildew caused by *Blumeriagraminis*f.sp. *tritici* (**Côté-Beaulieu et al. 2009; De Curtis et al. 2012**), Septoria leaf blotch (**Rodgers-Gray and Shaw 2000**), leaf blast caused by *Pyricularia oryzae* (**Pagani et al., 2014**), leaf rust caused by *Puccinia triticina* and yellow spot caused by *Drechslera tritici-repentis* (**Filho et al. 2013**), eyespot caused by *Oculimaculayallundae* (**Rodgers-Gray and Shaw 2004**), and spot blotch *Bipolarissorokiniana* (Domiciano et al., 2013). Therefore, a nano level of (Si) could increase the positive influence on plant growth and resistance and suppress plant pathogenic fungi. Similarly, **Suriyaprabha (2014)** indicated that SiO2 NPs are conducted as an alternative potent antifungal agent against phytopathogens. Thus, this study aims to evaluate the efficiency of SiO2 NPs and orange oil as safe alternatives to synthetic fungicides against leaf rust disease under field conditions. Also, assess their impacts on some biochemical targets and the crop yield.

Materials and Methods

Chemicals used

- Fungicide: Crwan® 25% EC ($30cm^3$/100L H2O) (Common name: propiconazole) was obtained from Central Agricultural Pesticides Laboratory (CAPL), ARC, Egypt.
- Orange oil NPs and bio and chemi-silica NPs were obtained from Sakha Agricultural Research Station, Kafr El-Sheik governorate, Egypt.

The characterisation of nanoparticles was described in our previous study by **Masoud et al. (2022a)** and used in the current study.

Field assessments

Experimental design

The present study was conducted at El-Gemmeiza Agricultural Research Station, El-Gharbya governorate, Egypt, during the 2022 and 2023 wheat growing seasons. A randomized complete block design with three replicates evaluated seven treatments plus control (untreated plants) for the severity of wheat yield and leaf rust disease. The area of each plot was 1.5 × 2.0 m^2, containing 5 rows 2.0 m long and 30 cm apart. Wheat seeds (Gemmeiza-7) of the tested materials were sown in the last week of November. The highly susceptible wheat genotype (Morocco) was shown on the border rows of the experimental area for the early development and spread of disease. Artificial inoculation of leaf rust was carried out during mid-February to create leaf rust epidemics. The cultural practices were performed as recommended. Different applications of SiONPs, orange oil NPs, and fungicides have been applied at wheat plants' 7-8 leaf growth stage. All applications were done three times for three weeks as foliar spray.

Disease assessment &yield parameters

Once rust symptoms were sufficiently matured and the spreader plants were 50% infected, leaf rust infections were examined. At weekly intervals, adult plants' leaf rust response data were graded four times as rust severity using Cobb's scale modified by **Peterson et al. (1948).** According to **Roelfs et al. (1992)**, plant response was expressed in five infection types:
- Immune (0): No uredia or other macroscopic infection indication
- Resistant (R): Small uredia surrounded by necrosis
- Moderately Resistant (MR): Small to medium uredia surrounded by chlorosis or necrosis
- Susceptible (S): Large uredia without chlorosis or necrosis
- Moderately Susceptible (MS): Medium-sized uredia with chlorosis

Other data recorded included the area under the disease progress curve (AUDPC), the number of grains, and grain weight per spike (g).
The AUDPC was calculated using the following formula:
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AUDPC = D [½ (Y1 + Yk) + (Y2 + Y3 +…… + Yk-1)]
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Where:
- D = days between two consecutive records (time intervals)
- Y1 + Yk = Sum of the first and last disease scores
- Y2 + Y3 +…….. + Yk-1 = Sum of all in-between disease scores

The area under the disease progress curve was classified into three categories based on their values:

- Score 1: The lowest AUDPC values ranged from 0 to 49 and referred to race-specific resistance
- Score 2: The moderate AUDPC values are less than 300 and refer to partial resistance (slow rusting resistance)
- Score 3: The high AUDPC values of more than 300 refer to fast rusting (highly susceptible wheat variety) (**El-Orabey et al. 2020**).

Laboratory assessments

Scanning electron microscope examination

The scanning electron microscope (SEM) was utilized to examine the effects of the applied treatments on the development of spores and the growth of P. triticina on wheat leaves. As described by **Harley and Fergusen (1990), sample preparation for SEM examination was carried out**. Using the JEOL model (SEM, Quanta FEG250, National Research Centre, Cairo, Egypt), interaction sites (spots) were noted, and disc blocks of 1 cm^2 were obtained for SEM observation. Changes in the morphological fungal structures between treated and untreated samples were examined and photographed.

Determination of chlorophyll

An aliquot (0.25 g) of wheat leaves was homogenized with 5 ml of acetone 80% using a hand glass homogenizer and filtered using Whatman filter paper. Then, the filtrate was completed to a volume of 50 ml with acetone and measured at a spectrophotometric instrument at 663 and 645 nm, according to **Grodzinsky and Grodzinsky (1973).** Chlorophyll a, b, and total (mg chlorophyll/g fresh weight) were calculated using the following equations:

- Chlorophyll a (Ch a) = $((12.7 \times O.D663) - (2.69 \times O.D645)) \times 0.2$
- Chlorophyll b (Ch. b) = $((22.9 \times O.D645) - (4.68 \times O.D.663)) \times 0.2$
- Total Chlorophyll (Ch T) = Ch a + Ch b

Determination of carotenoids

Fresh wheat leaves (0.25 g) were homogenized with acetone until the leaves were decolourized entirely and the extract was filtered. Then, the filtrate was completed to 50 ml with acetone, and a spectrophotometer measured the absorbance (A) at 450 nm according to the method of **Villanueva et al. (1985).**

Determination of protein content

The protein content was determined according to the method of **Maehr et al. (2016).** Wheat leaves (0.5 g) were homogenized with 30 ml of 0.1 M sodium hydroxide in 3.5% sodium chloride. The homogenates were incubated for 90 min at 60°C before centrifugation for 30 min at 6000 rpm under cooling. After that, the extracts were diluted to 1 ml with H2O and 0.9 ml of solution A before incubating for 10 min at 50°C. Then, 1 ml of solution B was added and left for 10 min. Finally, 3 ml of solution C was added before incubation for 10 min at 50°C. The absorbance was measured at 650 nm.

Determination of total phenolic content

Wheat leaves (2 g) were homogenized in 80% ethanol and centrifuged at 10,000 rpm for 15 min under cooling, and the supernatant was saved. The residue was again extracted twice with 80% ethanol, and the supernatants were pooled and evaporated to dryness. After that, it was dissolved in 5 ml of distilled water. Hundred µl of the extract was added to 0.5 ml of Folin-Ciocalteu reagent and 3 ml of water. After 3 min, 2 ml of 20% sodium carbonate was added, and then the absorbance was measured at 650 nm according to the method of **Singleton et al. (1999)**.

Determination of catalase activity

Determination of catalase (CAT) activity was done by homogenizing 1 g of wheat leaves with 100 mM phosphate buffer (pH 7.5), 1% PVP-40, and 1 mM EDTA. Afterwards, the homogenates were centrifuged at 4500 rpm and 5˚C for 15 min. The supernatants were collected and centrifuged at 10,000 rpm for 10 min. The activity was measured at 240 nm and expressed as U/mg protein (**Halka et al. 2019**).

Polyphenol oxidase (PPO)

To determine Polyphenol oxidase (PPO) activity, the wheat leaf sample was ground with 0.2 mM phosphate buffer at pH 7. The extract was transferred to a volumetric flask, and 0.05 mM phosphate buffer was added. After that, it was kept at 4°C for 2 h. The extract was mixed with a catechol solution (0.07 mM) and phosphate buffer solution (0.05 mM), and then the absorbance was measured at 420 nm.

Statistical analysis

The experiment was set up in a completely randomized design. The obtained data were analyzed using Analysis of Variance (ANOVA). The analysis was done using Costat 6.3111 software 1998-2005, and Duncan's multiple range test at $P < 0.05$ level was used for means separation (**Winer 1971**).

Results and discussion

Disease and yield assessments

Effects of different applications to control infested leaf rust in wheat during the growing seasons 2022 and 2023. The data presented in (Table 1) showed that all treatments resulted in a significant decrease in leaf rust disease severity compared to untreated plants. Moreover, untreated wheat plants significantly reduced grain yield compared to treated plants (Figure 1). Among the treatments, the fungicide Crwan® demonstrated the highest effectiveness, with mean values of 95.38% for disease control, an area under the disease progress curve (AUDPC) of 44, and 74.33 grains per spike with a grain weight of 3.27g. The bio-synthesized nano-silica at a concentration of 400 ppm exhibited an efficiency of 84.62%, an AUDPC of 160, 71.67 grains per spike, and a grain weight per spike of 2.98g. On the other hand, the nano-orange oil at a concentration of 200 ppm showed a lower reduction in disease severity with an efficiency of 36.54%, an AUDPC of 625, 55.67 grains per spike, and a grain weight per spike of 2.48g. These findings are consistent with the results obtained by **Lamsaletal. (2011)**, who found that using silver NPs (AgNPs) reduced disease severity in pumpkin and cucumber leaves. **Taha et al. (2020)** also discovered that treating lettuce plants with aqueous extracts of moringa, neem, basil, garlic, and the fungicide DiathineM-45® significantly decreased disease incidence and severity compared to untreated plants.

Table 1. Effect of different nano-materials on leaf rust in the wheat during 2022 and 2023 growing seasons.

Treatment	Disease Severity %	Efficiency %	AUDPC
Crwan®	4.00	95.38	44.00
Nano-bio silica (400 ppm)	13.33	84.62	160.00
Nano-chemical silica (400 ppm)	16.67	80.76	210.00
Nano-bio silica (200 ppm)	21.66	74.99	245.00
Nano-chemical silica (200 ppm)	31.67	63.46	355.00
Nano orange oil (400 ppm)	41.66	51.92	465.00
Nano orange oil (200 ppm)	55.00	36.54	625.00
Control	86.67	-	940.00
F test	**	-	**
LSD 0.05	14.785	-	3.782

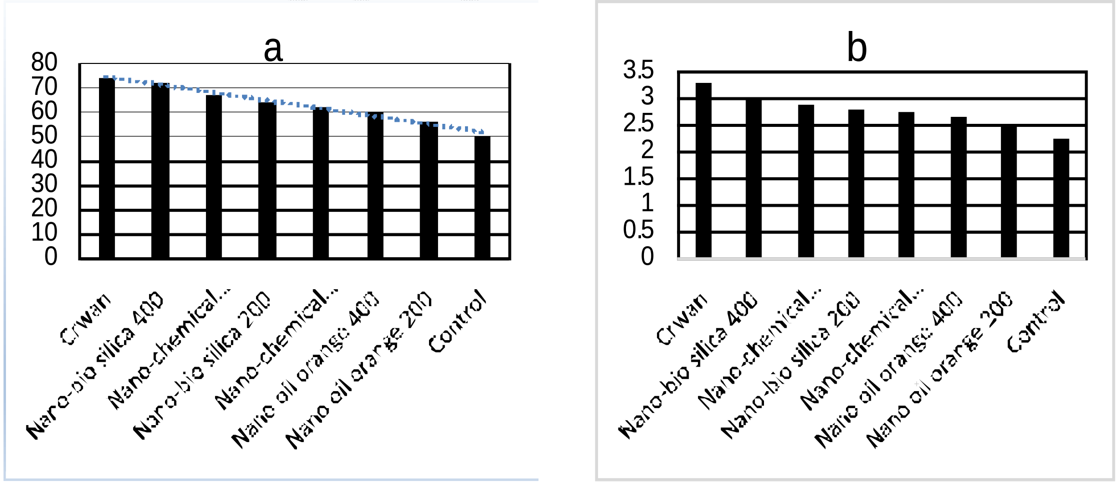

Figure 1. Effect of different treatments on (a) number of grains and (b) grain weight /spike (g) during the growing seasons of 2022 and 2023.

Scanning Electron Microscope (SEM) examination of the interaction among the most promising treatments and *Pucciniatriticina* on leaves of wheat

Several fungal morphological characteristics were examined from the leaf rust spots on the treated plants, compared to infected-untreated plants (control).

The investigation focused on the growth density of conidiophores and the disintegration of mycelium and conidia as key fungal morphological traits. The results revealed that the density of fungal mycelium was significantly reduced by the fungicide Crwan® (Figure 2A), particularly on leaves treated with nano orange oil at a concentration of 200 ppm (Figure 2B). Moreover, the ability of the phytopathogenic fungus to produce conidiophores and conidia was impaired, with Pucciniatriticina exhibiting a lower production of conidia. Furthermore, plasmolysis and breakdown of *Puccinia triticina's* mycelium and conidia were observed. Interestingly, on the treated leaves, conidia, mycelium, and conidiophores showed signs of incompleteness and exhibited twisted forms during their formation.

An important observation is the disappearance of most stomata in the wheat leaf under fungal infection in the control group (Figure 2C). Conversely, in the remaining treatments, the appearance of stomata was observed.

This observation highlights the impact of fungal infection on stomatal presence and emphasizes the potential influence of the tested treatments on this phenomenon.

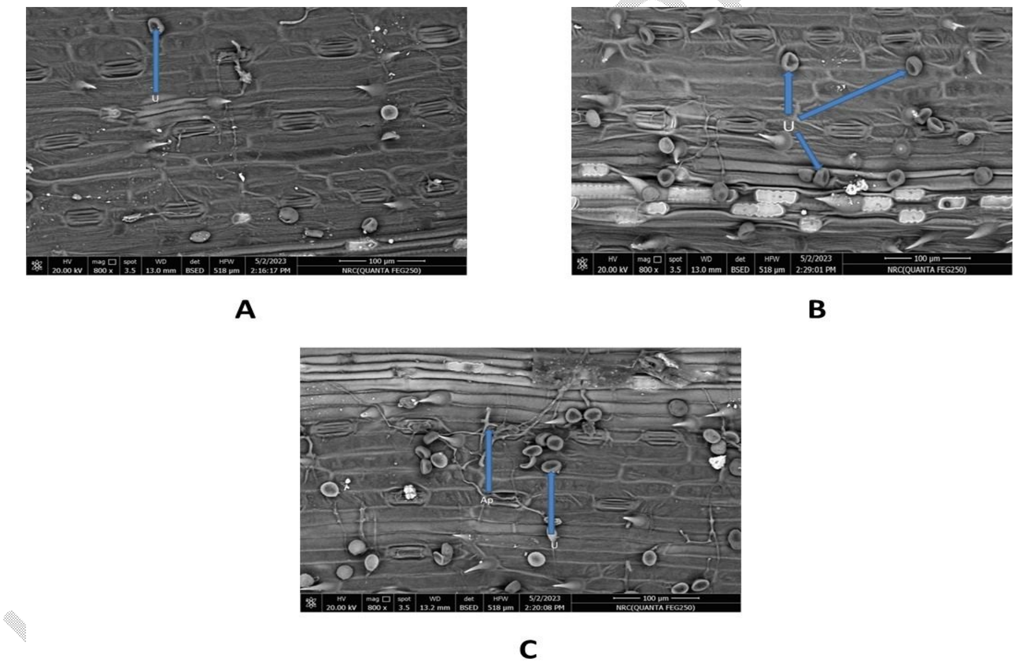

Figure 2. Micrograph of scanning electron microscopy findings that are promising foliar application on the wheat, (A) Crwan$^{®}$, (B) nano orange oil (200 ppm), and (C) Control (untreated), which visualized at 800X.

Effect of the treatments on carotenoid and chlorophyll contents in wheat leaves growing under field conditions during winter 2022 and 2023.

The data from the analyses conducted on wheat leaves revealed a diverse range of carotenoid and chlorophyll content, including chlorophyll a, b, and total chlorophyll. The measurements were performed on fresh leaves and are presented in Figure 3. Furthermore, the results demonstrated that applying different treatments impacted the concentrations of carotenoids and chlorophyll in the wheat leaves.

The highest carotenoid content was observed after the application of Crwan® and bio-synthesized nano-silica at a concentration of 400 ppm. Conversely, the lowest carotenoid content was recorded in the treatment involving nano-orange oil at a concentration of 200 ppm. It is worth noting that chlorophyll concentration was higher than that of carotenoids in all treatments, consistent with the findings of **Lopez-Ayerra et al. (1998**) and **Mitić et al. (2013).** Additionally, "Mancozeb" had a less pronounced effect on the chlorophyll and carotenoid content, suggesting their relatively stable photosynthetic characteristics (**Garcia et al. 2002**).

In contrast, **Saladin et al. (2003**) reported decreased pigment content following treatment with fludioxonil and carbendazim. These findings align with our observations of reduced chlorophyll content in infected plants, such as lime crops (**Zafari et al. 2012**), Chinese jujube (**Liu et al. 2016**), and lettuce leaves (**Akkurak et al. 2022**). Fatma &Nafady (2015) also demonstrated a significant enhancement in chlorophyll and carotene contents in wheat leaves treated with AgNPs.

These findings contribute to understanding the variations in carotenoid and chlorophyll content in wheat leaves under different treatments.

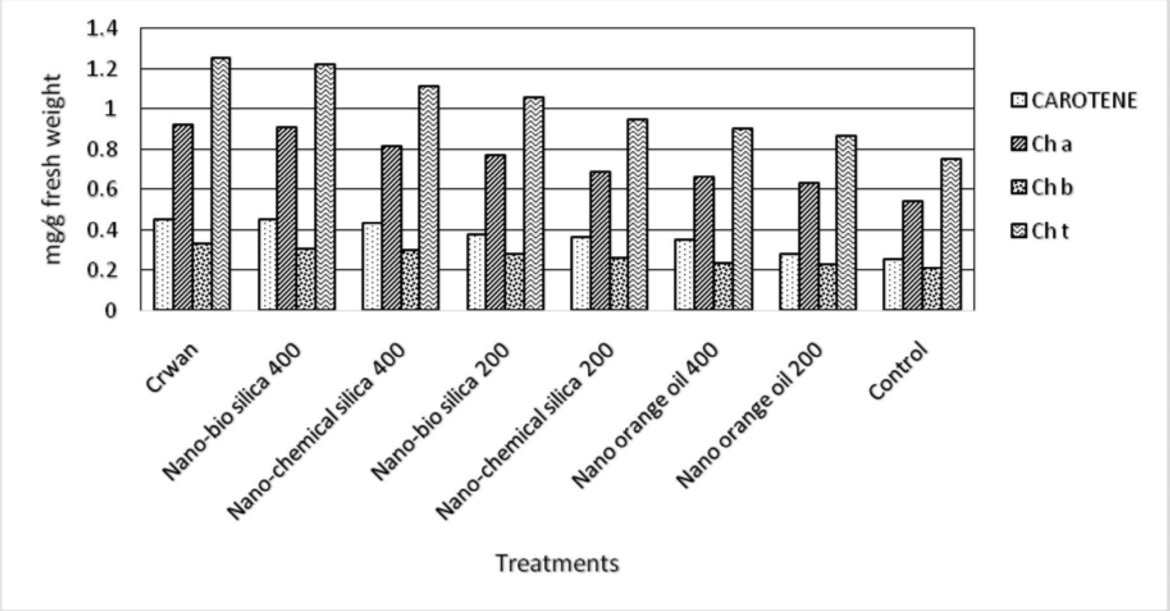

Figure 3. Effect of the treatments on carotenoid and chlorophyll contents during 2022 and 2023 seasons.

Effect of different applications on protein and phenolic contains in wheat leaves growing under field conditions during 2022 and 2023 seasons.

Table (2) presents the protein analysis results in the wheat leaf samples. A common observation was a decrease in protein content in most diseased plants. The soluble protein content in the leaves was significantly lower in untreated plants compared to the treated ones. Among the different applications, the Crwan® treatment exhibited the highest protein content, followed by the biosynthesized nano-silica at a concentration of 400 ppm. Wheat treated with various applications showed slightly higher protein content than the control group. These findings suggest that adequate moisture levels may enhance the effectiveness and profitability of these treatments.

Similar results were reported for protein deficiency in infected lettuce leaves by **Akkurak et al. (2022**) and **Nasir et al. (2017**), who observed a decrease in protein content in infected chickpea plants. **Masoud et al. (2022b**) found that spraying potato plants with Bio-Arc increased protein content.

Table 2 also presents the phenolic content in wheat leaves after treatment with nano-materials. Overall, all treatments significantly impacted the total phenolic content in wheat compared to the control group (untreated plants). The statistical analysis revealed higher concentrations of total phenols in wheat treated with Crwan® and biosynthesized nano-silica at a concentration of 400 ppm. Conversely, the lowest concentration of total phenols was observed in wheat treated with nano-orange oil at a concentration of 200 ppm.

Phenolic acids are considered secondary metabolites and act as natural antioxidants in plants. These compounds possess various biological activities, including anticancer, antioxidant, cytotoxic, antidepressant, and anti-inflammatory properties (**Ghasemzadeh and Ghasemzadeh 2011**). Moreover, increased levels of polyphenolic compounds can contribute to the strengthening of cell walls, which play a crucial role in protecting plants against microbial penetration (**Wulanjari et al., 2020**). **Sularz et al. (2021**) also reported increased polyphenolic compound concentration in lettuce leaves after applyingiodosalicylic acid.

These findings shed light on the protein and phenolic content variations in wheat leaves under different treatments, emphasizing the potential benefits of specific applications in enhancing these parameters.

Table 2. Effect of treatments on total protein and phenol in the wheat leaves growing under field conditions during 2022 and 2023 seasons.

Treatments	Total protein (mg/g)	Total phenol (mg/g fresh weight)
Crwan®	3.187	3.090
Biosyntheziednano-silica(400 ppm)	3.127	3.047
Chemisyntheziednano-silica(400 ppm)	3.050	2.883
Biosyntheziednano-silica(200 ppm)	2.857	2.857
Chemisyntheziednano-silica(200 ppm)	2.683	2.647
Nano orange oil (400 ppm)	2.653	2.640
Nano orange oil (200 ppm)	2.247	2.443
Control	2.067	2.170
F test	**	**
LSD 0.05	0.2114	0.1642

Effect of treatments on catalase (CAT) and polyphenol oxidase (PPO)activity in the wheat leaves growing under field conditions during 2022 and 2023 growing seasons.

Table (3) displays the enhanced activity of enzymes in wheat leaves following various treatments. The activity of CAT (catalase) and PPO (polyphenol oxidase) enzymes significantly increased with different applications. Among the treatments, the highest activity of CAT and PPO enzymes was observed in wheat leaves treated with Crwan®, followed by biosynthesized nano-silica at a concentration of 400 ppm, compared to the untreated plants. On the other hand, wheat plants treated with nano-orange oil at a concentration of 200 ppm exhibited the lowest activity of these enzymes.

The activity of the CAT enzyme was found to elevate in response to pathogen attacks in plants (**Magbanua et al. 2007**). CAT is an enzyme capable of protecting biological systems against free radical attacks (**Ighodaro and Akinloye 2018**) by reducing H2O2 into H2O and O2 (**Veronica et al. 2017**). In a similar context, the application of iodide increased CAT activity in lettuce (**Blasco et al. 2008 and 2011**). **Lei et al. (2008**) observed that TiO2 nanoparticles (NPs) decreased oxidative damage by increasing the activity of superoxide dismutase (SOD), ascorbic peroxidase (APX), and CAT in spinach chloroplast. **Krishnaraj et al. (2012**) reported increased activity of POX and CAT enzymes in leaf samples of plants treated with silver nanoparticles (AgNPs). Furthermore, **Farrag (2015**) observed increased CAT activity in tested plants after treatment with AgNPs.

These findings highlight the impact of different treatments on enzyme activity in wheat leaves, particularly the enhanced activity of CAT and PPO enzymes.

Table 3. Effect of treatments on catalase (CAT) and polyphenol oxidase (PPO) in the wheat leaves growing under field conditions during the 2022 and 2023 seasons.

Treatments	Catalase (CAT) (U/mg protein)	Polyphenol oxidase (PPO) (ΔE 420 nm min-1 g-1)
Crwan®	0.201	0.790
Biosyntheziednano-silica(400 ppm)	0.194	0.785
Chemisyntheziednano-silica(400 ppm)	0.189	0.757
Biosyntheziednano-silica(200 ppm)	0.186	0.755
Chemisyntheziednano-silica(200 ppm)	0.184	0.741
Nano orange oil (400 ppm)	0.181	0.733
Nano orange oil (200 ppm)	0.175	0.726
Control	0.165	0.685
F test	**	**
LSD 0.05	9.41	0.0208

CONCLUSION

Spraying wheat leaves with SiONPs and nano-orange oil has decreased the infection of leaf rust disease. These different applications have had significant effects in reducing disease severity and enhancing the contents of chlorophyll, carotenoids, phenolics, and proteins compared to the control group. Moreover, the activities of CAT and PPO enzymes increased in the treated samples compared to their respective controls.

Based on these findings, it can be concluded that these nano-materials are highly effective in combating wheat rust disease. However, further investigations are urgently needed to establish their practices as eco-friendly alternatives.

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