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Biochemical Responses of Wheat Leaf Rust Disease by Using Silica and Orange Oil Nanoparticles

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Authors' contributions

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

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ABSTRACT

The potential antifungal activity of different applications of biologically and chemically 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.

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Finally, the fungicide, Crwan® and the bio-synthesized silica nanoparticles (400 ppm), yielded the best results in our study compared to other applications, while orange oil NPs (200 ppm) were the least effective.

Keywords: Wheat; leaf rust; silica nanoparticles; orange oil NPs; fungicide.

1. INTRODUCTION

Wheat (*Triticum aestivum* L.) is an important cereal crop worldwide and a staple food for about one-third of the world's population. Leaf rust disease is caused by the fungus *Puccinia triticina* f.sp. *tritici* causes yield loss due to decreased kernel numbers per head and reduced kernel weights [1,2,3,4,5,6,7,8,9,10,11,12,13,14,15]. Leaf rust causes significant crop losses associated with geographical regions and locations [16,17].

Citrus plants belong to the *Rutaceae* family, which has several subtypes of plants, as mandarins, oranges, lemons, limes, grapefruits, and citrons. According to FAO, citrus wastes contain high-quality fiber, pectin, and their composition of many valuable bioactive compounds as polyphenols, hesperidin, carotenoids, flavonoids, and essential oils (EOs). Citrus have been used in different industrial products as food, cosmetics, pharmaceutical, and beverage [18,19]. Hesperidin (3', 5, 7-trihydroxy-4'-methoxy flavanone-7-6-O-α-L-rhamnosyl-D-glucose) is a flavonoid by-product found in citrus production, basically in lemon and sweet orange. Many reports showed that hesperidin displays different pharmaceutical effects as antioxidant, anti-inflammatory and anti-allergic [20]. And also, it displayed cytotoxic activity against several rat model carcinogenesis, including the esophagus, tongue, urinary bladder and colon [21]. Citrus Eos, and flavonoids are widely known for their beneficial effects in possessing several biological activities, as antimicrobial, antioxidant and cytotoxic properties. They are used in food additives, and cosmetics [22]. These oils are studied for their potential uses in food industry. Their composition is a mixture of oxygenated compounds, hydrocarbons, and non-volatile residues, including sesquiterpenes, terpenes, aldehydes, esters, alcohols, and sterols [23,24].

Nano-technology is an important technique of modern science that has contributed to every part of life. Nano-particles have a size range from 1 to 100 nm [25,26], physicochemical characters that differ from bulk materials, could be used as and as plant protection products, growth stimulator, and help to improve nutrients [27]. In recent years, nano-materials (NMs) have been investigated independently for their biological activities and pharmaceutical. Their potency is independent of size, physical properties, stability, surface charges, and others [28]. They are employed in a wide range of applications in different science, such as agriculture, biomedical, environmental remediation, and electronic information technology applications [29]. Several studies have confirmed the ability of nanomaterials to improve seed germination and growth [30,31,32]. Nanoparticles are environmentally friendly and have recently been used as a safe alternative to chemical fertilizers and pesticides in agriculture farms to reduce infectious diseases and improve crop yield. Positive effects of several nanoparticles, *i.e*. zinc oxide (ZnO), titanium dioxide (TiO2), chitosan (CS) and nickel (Ni) on the growth of wheat seedlings were confirmed [33,34].

Silicone cation (Si) can stimulate the resistance mechanisms of plants against biotic agents and improve plant growth [35,36] and abiotic stress [31,32]. Also (Si) can inhibit several plant diseases, as brown spot, stem rot, bacterial blight, leaf scald, grain discolouration, sheath blight, leaf and panicle blast in rice, as well as powdery mildew in cucumber and wheat [37]. In wheat crop, (Si) significantly suppressed many fungal diseases as powdery mildew caused by *Blumeria graminis* [35,38], Septoria leaf blotch and eyespot caused by *Oculimacula yallundae* [39], leaf blast caused by *Magnaporthe oryzae* [40], and spot blotch *Bipolaris sorokiniana* [41], leaf rust disease caused by *Puccinia triticina tritici* and yellow spot caused by *Drechslera tritici-repentis* [36]. Therefore, a nano level of Silicone could increase the positive influence on plant resistance and suppress plant pathogenic fungi and growth. Similarly, Suriyaprabha et al. [42] approved that SiO2 Nanoparticles are used as an alternative potent antifungal agent against phyto-pathogens. 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.

And also, assess their impacts on some biochemical targets and the crop yield.

2. MATERIALS AND METHODS

2.1 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 Agricultural Research Station, Sakha, Egypt.

The characterisation of nanoparticles was described in our previous study by Masoud et al. [43] and used in the current study.

2.2 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. Wheat seeds (Gemmeiza-7) of the tested materials were sown in the last week of November. The highly susceptible wheat genotype (Morocco cv.) was sown on the border of the experimental area for the development and spread of the 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 (200 and 400 ppm), 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 [44].

According to [45], 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 “area under disease progress curve” (AUDPC), the number of grains, and grain weight per spike (g).

The AUDPC was calculated using the following formula:

$$AUDPC = D\left[\tfrac{1}{2}(Y1 + Yk) + (Y2 + Y3 + \ldots\ldots + Yk\text{-}1)\right]$$

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) [10].

2.3 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 [46], sample preparation for SEM examination was carried out using the JEOL model (SEM, Quanta FEG250, National Research Centre, Cairo, Egypt), interaction spots were noted, and disc blocks of 1cm^2 were obtained for SEM observation. Changes in the morphological fungal structures between treated and untreated samples were examined and photographed.

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 [47].

Determination of chlorophyll: An aliquot (0.25g) of wheat leaves was homogenized with 5ml 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 [48]. Chlorophyll a, b, and total (mg chlorophyll/g fresh weight) were calculated using the following equations:

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

Determination of protein content: The protein content was measured according to the method of [49]. Wheat leaves (0.5g) were homogenized with 30ml of 0.1M 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 1ml with H2O and 0.9ml of solution A before incubating for 10min at 50°C. Then, 1ml of solution B was added and left for 10min. Finally, 3ml of solution C was added before incubation for 10min at 50°C. The absorbance was measured at 650nm.

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 [50].

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 determined at 240nm and expressed as U/mg protein [51].

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.

2.4 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 [52].

3. RESULTS

3.1 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 (Fig. 1). Among the treatments, the fungicide Crwan® demonstrated the highest effectiveness, with mean values of 95.38% for disease control, an area under disease progress curve (AUDPC) of 44, and 74.33 grains per spike with a grain weight of 3.27g. The biologically synthesized silica nanoparticles 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.

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
Biologically synthesized silica nanoparticles (400 ppm)	13.33	84.62	160.00
(400 ppm)	16.67	80.76	210.00
Biologically synthesized silica nanoparticles (200 ppm)	21.66	74.99	245.00
Chemically synthesized silica nanoparticles (200 ppm)	31.67	63.46	355.00
Orange oil nanoparticles (400 ppm)	41.66	51.92	465.00
Orange oil nanoparticles (200 ppm)	55.00	36.54	625.00
Control	86.67	-	940.00
F test	**	-	**
LSD 0.05	14.785	-	3.782

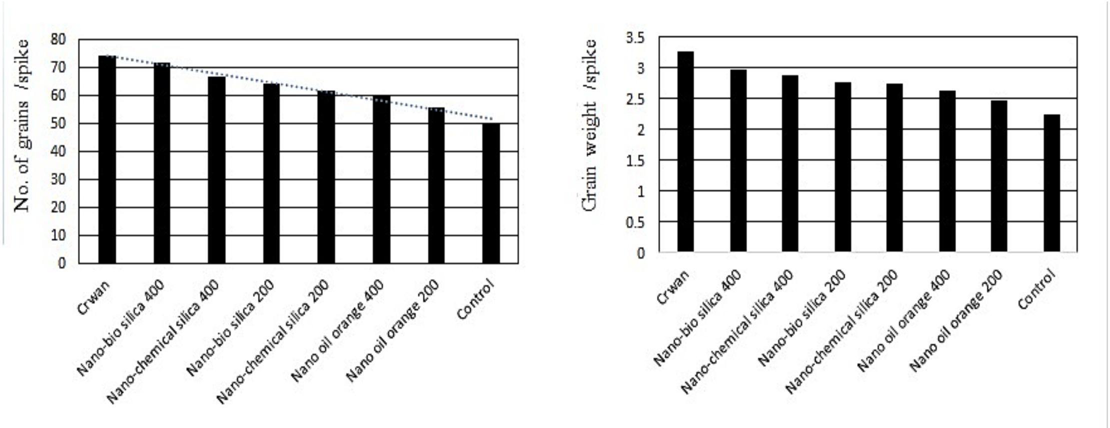

Fig. 1. Effect of different treatments on number of grains and grain weight /spike (g) during the growing seasons of 2022 and 2023

3.2 Scanning Electron Microscope

Examination of the interaction among the most promising treatments and *Puccinia triticina* on leaves of wheat: Different fungal morphological properities 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® (Fig. 2), particularly on leaves treated with orange oil nanoparticles at a concentration of 200 ppm. Moreover, the ability of fungus to produce conidiophores and conidia was impaired, with *Puccinia triticina* 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. 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.

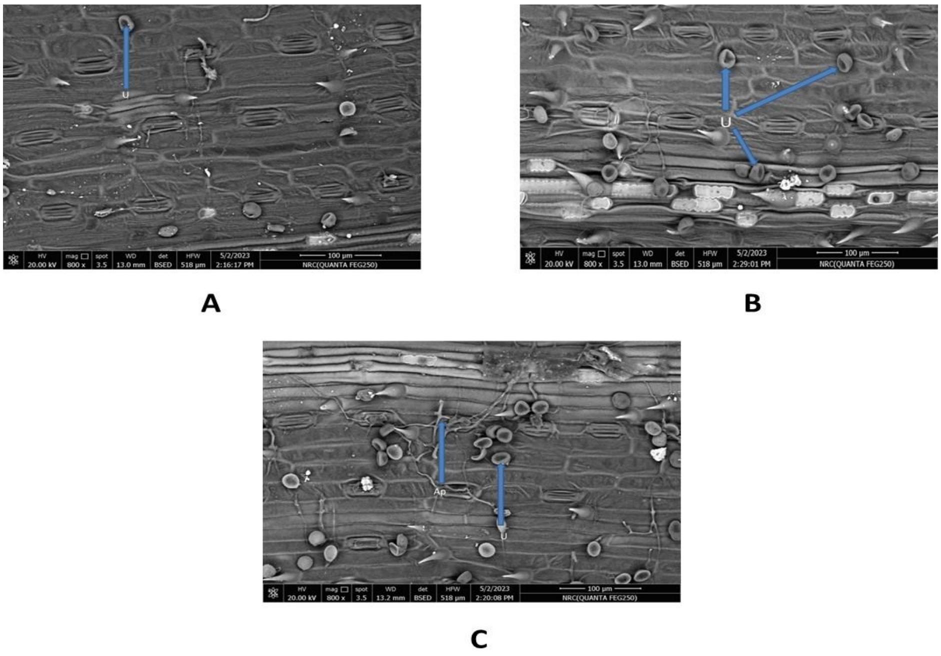

Fig. 2. Micrograph of scanning electron microscopy findings that are promising foliar application on the wheat, (A) Crwan®, (B) orange oil nanoparticles (200 ppm), and (C) Control (untreated), which visualized at 800X

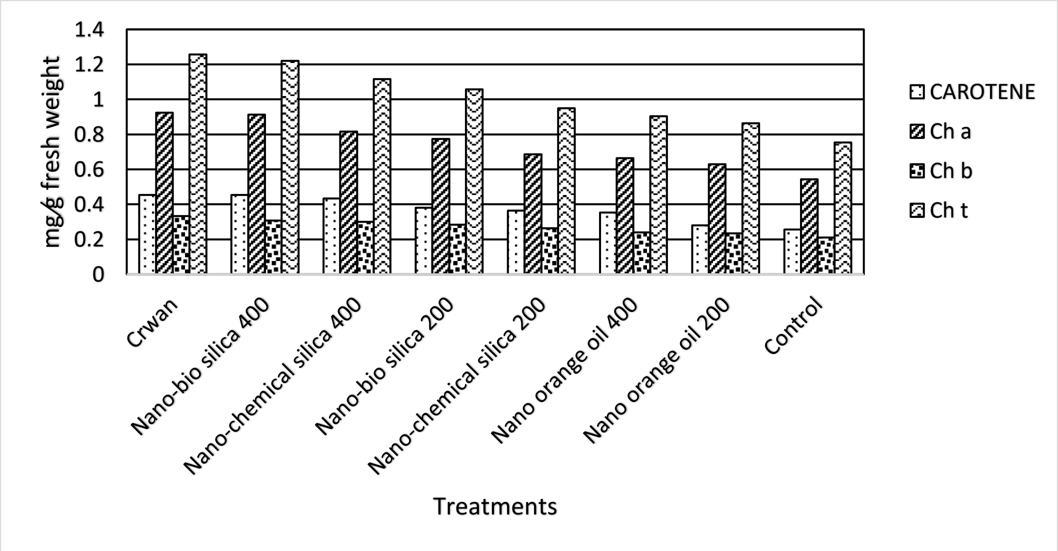

Fig. 3. Effect of different treatments on carotenoid and chlorophyll contents during 2022 and 2023 seasons

Effect of different 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 (Fig. 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 biologicaly synthesized silica nanoparticles 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.

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 lower in control compared to the treated ones. Among the different applications, the Crwan® treatment exhibited the highest protein content, followed by the biologically synthesized silica nanoparticles at a concentration of 400 ppm. Wheat treated with various applications showed slightly higher protein content than the control. 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 (untreated plants). The statistical analysis revealed higher concentrations of total phenols in wheat treated with Crwan® and biologically synthesized silica nanoparticles at a concentration of 400 ppm. Conversely, the lowest concentration of total phenols was observed in wheat treated with orange oil nanoparticles at a concentration of 200 ppm.

Effect of different 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 biologically synthesized silica nanoparticles 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.

Table 2. Effect of different 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
Biologically syntheziedsilica nanoparticles (400 ppm)	3.127	3.047
Chemically syntheziedsilica nanoparticles (400 ppm)	3.050	2.883
Biologically synthezied silica nanoparticles (200 ppm)	2.857	2.857
Chemically synthezied silica nanoparticles (200 ppm)	2.683	2.647
Orange oil nanoparticles (400 ppm)	2.653	2.640
Orange oil nanoparticles (200 ppm)	2.247	2.443
Control	2.067	2.170
F test	**	**
LSD 0.05	0.2114	0.1642

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

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

4. DISCUSSION

All treatments resulted in a significant decrease in leaf rust disease severity compared to untreated plants. Among the treatments, the fungicide Crwan® and the bio-synthesized silica nanoparticles at a concentration of 400 ppm demonstrated the highest effectiveness, for disease control, grains per spike and a grain weight. On the other hand, the nano-orange oil at a concentration of 200 ppm showed a lower reduction in disease severity, grains per spike, and a grain weight per spike. These findings are consistent with the results obtained by [53], who found that using silver NPs (AgNPs) reduced disease severity in pumpkin and cucumber leaves. Taha et al. [54] 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.

The 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 silica nanoparticles at a concentration of 400 ppm. It is worth noting that chlorophyll concentration was higher than that of carotenoids in all treatments, consistent with the findings of [55, 56]. Additionally, "Mancozeb" had a less pronounced effect on the chlorophyll and carotenoid content, suggesting their relatively stable photosynthetic characteristics [57]. In contrast [58] 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 [59], Chinese jujube [60], and lettuce leaves [61]. Fatma and Nafady [62] 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.

The soluble protein content in the leaves was significantly lower in untreated plants compared to the treated ones. The Crwan® treatment exhibited the highest protein content, followed by the biosynthesized silica nanoparticles at a concentration of 400 ppm. Wheat treated with various applications showed slightly higher protein content than the control group. Similar results were reported for protein deficiency in infected lettuce leaves by [63,64], who observed a decrease in protein content in infected chickpea plants. Masoud et al. [65] found that spraying potato plants with Bio-Arc increased protein content.

Meanwhile, all treatments significantly impacted the total phenolic content in wheat compared to the control group. The higher concentrations of total phenols in wheat treated with Crwan® and biosynthesized silica nanoparticles at a concentration of 400 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 [66]. 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 [67]. Sularz et al. [68] also reported increased polyphenolic compound concentration in lettuce leaves after applying iodo-salicylic 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.

The activity of CAT (catalase) and PPO (polyphenol oxidase) enzymes significantly increased with different applications. The highest activity of CAT and PPO enzymes was observed in wheat leaves treated with Crwan®, followed by biosynthesized silica nanoparticles 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 [69]. CAT is an enzyme capable of protecting biological systems against free radical attacks [70] by reducing H2O2 into H2O and O2 [71]. In a similar context, the application of iodide increased CAT activity in lettuce [72,73]. Lei et al. [74] observed that TiO2 nanoparticles (NPs) decreased oxidative damage by increasing the activity of superoxide dismutase, ascorbic peroxidase (APX), and CAT in spinach chloroplast. Krishnaraj et al. [75] reported increased activity of POX and CAT enzymes in leaf samples of plants treated with silver nanoparticles (AgNPs). Furthermore, Farrag [76] 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.

5. CONCLUSION

Spraying wheat leaves with SiONPs and orange oil nanoparticles has decreased the infection of leaf rust disease. These different applications have significant effects on reducing disease severity and enhancing the contents of chlorophyll, carotenoids, phenolics, and proteins compared to the control. 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 biologically synthesized silica nanoparticles at a concentration of 400 ppm are highly effective in combating wheat rust disease. However, further investigations are urgently needed to establish their practices as eco-friendly alternatives.

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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