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**ORIGINAL PAPER**

# Nanochitosan and Water-Soluble Vitamins Induce Resistance to Leaf Rust and Related Metabolism in Wheat

**Elkhwaga, A.A.; Eldakar, H.A.M.; Alkollaly, A.M.; Draz, I.S. and Adbelghany, R.E.A.**

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## ABSTRACT

Inducing resistance by prior application of nanochitosan, chitosan, and vitamins B3 (nicotinic acid), B9 (folic acid) and C (ascorbic acid), to counteract wheat leaf rust caused by *Puccinia triticina* Eriks. was investigated in two field trials during 2021-2022 and 2022-2023 growing seasons. The tested materials were sprayed pre-infection with the disease on a susceptible wheat cultivar (Gemmiza-7). The response of the treated plants changed to moderately resistant (MR) by nanochitosan and folic acid (Vit. B9), while they changed to moderately susceptible (MS) by nicotinic acid (Vit. B3) and ascorbic acid (Vit. C) as compared to the susceptible response in the fungicide (propiconazole 25%) and untreated control. All treatments offered good levels of disease protection, which reached 95.0% in the case of nanochitosan and folic acid comparable to the fungicide (propiconazole 25%), being 93.75%. Also, they significantly reduced the average coefficient of infection (ACI). The best treatments were nanochitosan and folic acid, since they reduced ACI to 4 comparable to the fungicide (propiconazole 25%), being 5 and untreated control, being 80. In addition, these treatments resulted in the highest increase in grain yield components. Metabolic aspects analyses revealed a significant increase in peroxidase (POX) and catalase (CAT) antioxidant enzyme activity as well as total chlorophyll and total phenols due to the tested treatments. The use of chitosan nanoparticles and vitamins B3, B9 and C may be an environmentally friendly strategy to induce resistance against leaf rust of wheat.

**Keywords**: Wheat, *Triticum aestivum*, Leaf rust, *Puccinia triticina*, Nanochitosan, Vitamins, Metabolic aspects, Induced resistance

***Correspondence: Eldakar, H.A.M.**
**E-mail:** eldakar2009@yahoo.com
**Amal A. Elkhwaga**
**Hala A.M. Eldakar**
**Asmaa M. Alkollaly**
**Ibrahim S. Draz**
https://orcid.org/0000-0002-2581-3556
**Rady E.A. Adbelghany**
**Plant Pathology Research Institute, Agricultural Research Center, 12619, Giza, Egypt.**

## INTRODUCTION

Leaf rust (*Puccinia triticina* Eriks.), is one of the most important obstacles to achieving sustainable development goals in the production of wheat (*Triticum aestivum* L.) worldwide and in Egypt, which results in significant grain yield losses (Abdel-Hak *et al.*, 1980 and Huerta-Espino *et al.*, 2011). Leaf rust can reduce grain yield by 35–50% (McVey *et al.*, 2004; Germán *et al.*, 2007 and Draz *et al.*, 2015), while in regions where it is endemic, it can reduce yield by as much as 60% (Lesovoi *et al.*, 1981). Management of the disease usually relies on resistant cultivars (Draz and Abd-Ekrem, 2021) and the use of fungicides (Barro *et al.*, 2017). The fungus *P. triticina* can evolve into new virulent races, which can overcome the plant's defense. Additionally, fungicides' harmful effects on the environment are sharply rising every day. To reduce the use of synthetic fungicides, alternative strategies are being explored, with biochemical changes being one of the effective ones that integrate natural antifungal compounds. The use of biological treatments for plant diseases with bio-fungicides is strongly encouraged and advised due to the considerable environmental risk posed by chemical fungicides (Dubey *et al.*, 2008).

Induced resistance, which utilizes the plant's intrinsic defense system, is a prospective strategy for the management of plant diseases. Application of specific chemicals and pre-inoculation with pathogenic or non-pathogenic microorganisms can both systemically induce resistance in some susceptible plants (Kuc, 1982). Chitosan is a well-known biocontrol agent with nontoxic, biodegradable and biocompatible characteristics (Hassan and Chang, 2017). Instead of acting as a direct antimicrobial or poisonous agent, chitosan is frequently utilized in plant disease control as a potent elicitor (El Hadrami *et al.*, 2010). We still don't know how chitosan nanoparticles affect severe wheat rust diseases or how induced resistance works (Elsharkawy *et al.*, 2022). To reduce the usage of fungicides totally or partially, creative alternative control strategies

that combine safe and environmentally friendly practices, like chitosan nanoparticles, are needed. Vitamins can act as disease-resistance inducers that have attracted significant attention due to their safety and cost-effectiveness (Boubakri *et al.*, 2016). The present work mainly explored the effectiveness of nanochitosan, chitosan and water-soluble vitamins as elicitors of resistance against leaf rust of wheat and to determine the metabolic aspects involved in the induced resistance.

## MATERIALS AND METHODS

**Synthesis and characterization of chitosan nanoparticles:**

Chitosan (CS) was obtained from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). Chitosan solution (0.2% w/v) was prepared after dissolving CS powder in glacial acetic acid (1% w/v) overnight shaking at ambient temperature (25°C). Chitosan nanoparticles were prepared in the Department of Corn and Sugar Crops Diseases, Agricultural Research Center (ARC), Egypt, by using the method of ionic gelation (Rampino *et al*., 2013). The resultant suspension was then centrifuged for 10 minutes at 15,000 rpm before being roughly 28°C dried at room temperature (Ghadi *et al.*, 2014). The size and morphologies of nanoparticles were determined by transmission electron microscopy (TEM, JEOL JEM 1400) at the National Research Center, Cairo, Egypt. Size of nanoparticles in suspension was determined by Zeta sizer (nano series, Nano ZS, Malvern, UK) in the Regional Center for Food and Feed. ARC, Giza, Egypt. The used concentration of nanochitosan was 150 µg $mL^{-1}$ (150 ppm).

**Preparation of vitamins:**

Three water-soluble vitamins *i.e*., vitamin B3 (nicotinic acid) also called niacin, vitamin B9 (folic acid), and vitamin C (ascorbic acid) were obtained from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). Each vitamin was prepared by mixing 0.1 g of each ingredient with 1000 ml of distilled water to obtain a final concentration of 100 g $mL^{-1}$ (100 ppm).

**Application process:**

Five treatments *i.e.*, nanochitosan, chitosan, vitamin B3 (nicotinic acid), vitamin B9 (folic acid), and vitamin C (ascorbic acid) were used to study their potential contribution to induce resistance against wheat leaf rust under field conditions. Field tests were conducted throughout two growing seasons (2021/2022 and 2022/2023) at Gemmeiza Agricultural Research Station's Experimental Farm, ARC, Egypt. At a rate of 40 g $plot^{-1}$, grains of a susceptible wheat cultivar (cv. Gemmeiza-7) were planted in three rows 3 m long and 30 cm apart across three-by-two-meter plots. A randomized complete block design (RCBD) with three replicates was used to set up the experiment. Treatments were applied 1 day before inoculation. Using a hand sprayer to water the plants, treatments were administered to leaves at growth stage GS 37 (Zadoks *et al*., 1974). As a comparable control, the chemical fungicide propiconazole 25% EC was used (0.25 $cm^3$ $L^{-1}$). The untreated control plots received distilled water spraying. The artificial inoculation was performed the next day after application using a mixture of urediniospores of *P. triticina* isolates (Tervet and Cassel, 1951). The pathogen inoculum represented a mixture of leaf rust isolates collected from infected wheat fields in Egypt, to best evaluate treatment efficacy against a broad spectrum of races.

**Disease assessment:**

Rust scoring was done at GS 83 (Zadoks *et al*., 1974). Host response was scored as: resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible (S) according to Roelfs *et al*. (1992). Rust severity was evaluated using Cobb's scale modified by Peterson *et al*. (1948). The coefficient of infection (CI) was determined by multiplying disease severity and constant values of infection types (Saari and Wilcoxson, 1974). Constant values for infection types were utilized as follows: R (0.2), MR (0.4), MR-MS (0.6), MS (0.8), and S (1.0). The average coefficient of infection (ACI) was calculated as the mean of the CI values over both growing seasons. Disease protection (efficacy) of treatment was determined according to Rewal and Jhooty (1985) using the following equation:

$$\textbf{Disease protection \% = c-t/c × 100}$$

**Where:**

**c** = infection in untreated control

**t** = treatment infection.

**Yield assessment:**

Grain yield components were assessed at harvest based on the spike weight (g), the 1000-kernel weight (g), and the volume weight (g $L^{-1}$).

**Metabolic aspects assay:**

Metabolic aspects in leaf rust infected-wheat leaves treated with the tested materials before inoculation were assayed at the Integrated Control Research Department, Plant Pathology Research Institute, ARC, Giza, Egypt. The effects of the treatments on some biochemical components, such as antioxidant enzyme

activity, peroxidase (POX) and catalase, as well as chlorophyll and phenol contents, were studied in adult plant leaf samples. Sampling was done on the first, third-, and seventh-days post-inoculation (dpi). These samples represented five treatments *i.e.*, nanochitosan, chitosan, vitamin B3 (nicotinic acid), vitamin B9 (folic acid), and vitamin C (ascorbic acid) plus fungicide control (propiconazole 25%) and untreated control.

**Peroxidase activity assay:**

Peroxidase was assayed in a spectrophotometer (model UV-160A, Shimadzu, Japan) according to the procedure proposed by Amako *et al*., (1994). The assay was performed at 25°C in 1.0 cm light path cuvette. The reaction mixture consisted of 1500 µL phosphate buffer, 1000 µL pyrogallol and 480 µL $H_2O_2$ solution. After mixing, the reaction was initiated by adding the enzyme extract (20 µL) and the increase in optical density at 430 nm against blank (without extract) was continuously recorded every minute (for 1 min). Enzyme activity was expressed as the increase in absorbance $min^{-1}$ $g^{-1}$ fresh weight.

**Catalase activity assay:**

Catalase activity was evaluated according to the method used by Aebi (1984). In which the disappearance of $H_2O_2$ in a reaction mixture containing 0.3 mL 3% $H_2O_2$, 2.5 mL of 0.05 M phosphate buffer (pH 7), and 2.5 mL of plant extract is gauged by the drop in absorbance at 240 nm.

**Chlorophyll content assay:**

The total contents of chlorophylls were assayed according to the method of Dere *et al*., (1998). Fresh leaves (0.1 g) were divided into tiny pieces (1 × 1 mm), submerged in 20 ml methanol (96%) for 24 h at 4 °C, and then filtered through Whatman 47 mm GF/C filter paper. The absorbance of each filtrate was evaluated in comparison to a blank methanol solution made of 96% at wavelengths of 666 nm and 653 nm. Chlorophyll contents were reported as mg $g^{-1}$ fresh weight according to Lichtenthaler and Wellburn (1983).

**Phenol content assay:**

The concentration of phenolic compounds was estimated using the Folin-Ciocalteu reagent (FCR) described by Kahkonen *et al*., (1999) with slight modification. In this, 1.0 ml oh FCR (10%) was added to 0.2 ml of methanolic extract (80%) of dried wheat leaves, then vortexed. Three min later, 0.8 ml of 7.5% (w/v) sodium carbonates was added to the mixture. The mixture was shaken, and incubated at room temperature for 30 min. At 765 nm, the absorption was measured. The concentration of phenolics was expressed as mg Gallic Acid Equivalents (GAE) per gram dry weight (g DW).

**Statistical analysis:**

The obtained data were analysed for ANOVA using the SAS v.22 Statistical Analysis System package (SAS Institute, Cary, NC, US). Means of data were separated at the least significant difference (LSD) test at $P \leq 0.05$ (Steel and Torrie, 1980).

## RESULTS

**Characterization of chitosan nanoparticles:**

Fig. (1 left) displays a typical Transmission Electron Microscope (TEM) micrograph of chitosan nanoparticles. The micrograph displays a homogeneous population of nanoparticles that have a variety of shapes, with most of them spherical and a few other types appearing sporadically oval. Average hydrodynamic diameters and particle size distribution (poly disparity indices) were measured using the zeta average diameter. The size distributions of nanoparticles in colloids, which ranged from 48.77 to 135 nm, are shown in Fig. (1 right). Most of them had a diameter of about 88 nm for all colloidal solutions. Whereas only 12% of all solutions have particles larger than 100 nm. The poly disparity index (PDI) showed a value of 0.87.

**Efficacy of nanochitosan, chitosan and vitamins against leaf rust of wheat:**

Data in Table (1) show that foliar spray of wheat plants (cv. Gemmeiza-7) with the 5 evaluated materials pre-infection with leaf rust offered effective protection against leaf rust under field conditions during 2021/22 and 2022/23 growing seasons. In both growing seasons, the response patterns of plants were changed from susceptible (S) in untreated control and fungicide to moderately resistant (MR) by application of nanochitosan and folic acid (Vit. B9), while they changed to moderately susceptible (MS) by application of chitosan, nicotinic acid (Vit. B3) and ascorbic acid (Vit. C). In the first season, nanochitosan and folic acid showed a response pattern of 10 MR and 20 MR, respectively, while they showed 10 MR for each in the second season. Nicotinic acid (Vit.B3) and ascorbic acid (Vit. C), each showed 10 MS, while chitosan showed 20 MS during both seasons. Average coefficients of infection (ACI) indicated highly significant differences among all treatments and untreated control,

while no significant differences were observed between the interaction of treatments and seasons. Foliar application of all tested materials significantly reduced the infection of leaf rust as ACI during both growing seasons. The best treatments were nanochitosan, which reduced CI value to 4 in both seasons, followed by folic acid with CI values of 8 and 4 in both seasons, respectively. Nicotinic acid and ascorbic acid ranked second (CI = 8), followed by chitosan (CI = 16) during both growing seasons. All treatments were comparable to the fungicide (propiconazole 25%) treatment (CI = 5) and the untreated control with a CI of 90 in the first season and 80 in the second season, respectively.

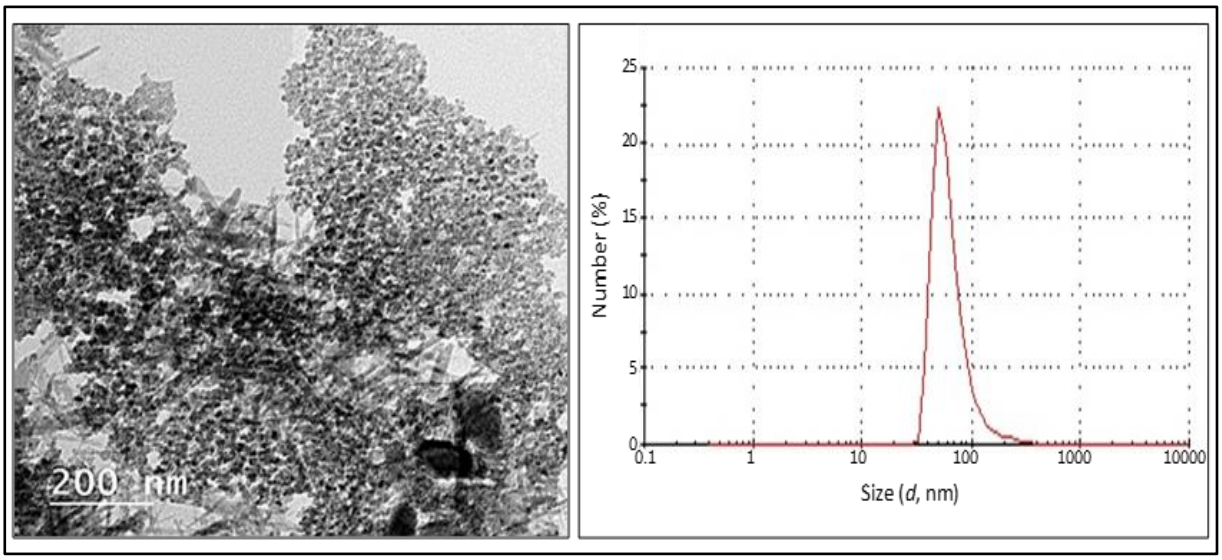


**Fig. (1): TEM image of chitosan nanoparticles (left) and Zeta sizer average diameter of chitosan nanoparticles (right).**

**Table (1): Effect of foliar application of nanochitosan, chitosan and vitamins (B3, B9 and C) on wheat leaf rust (cv. Gemmieza-7) under field conditions during 2021/22 and 2022/23 growing seasons.**

| Treatment | Host response | | Coefficient of infection (CI) | | ACI* |
|---|---|---|---|---|---|
| | 1st season | 2nd season | 1st season | 2nd season | |
| Nanochitosan | 10 MR | 10 MR | 4 | 4 | 4 |
| Chitosan | 20 MS | 20 MS | 16 | 16 | 16 |
| Nicotinic acid (Vit. B3) | 10 MS | 10 MS | 8 | 8 | 8 |
| Folic acid (Vit. B9) | 20 MR | 10 MR | 8 | 4 | 6 |
| Ascorbic acid (Vit. C) | 10 MS | 10 MS | 8 | 8 | 8 |
| Fungicide (propiconazole 25%) | 5 S | 5 S | 5 | 5 | 5 |
| Control (untreated) | 90 S | 80 S | 90 | 80 | 85 |
| LSD at 0.05 | | | | | |
| Treatments (T) | | | 6.54 | 6.72 | 6.03 |
| Seasons (S) | | | | | 3.22 |
| T × S | | | | | ns |

* ACI = average coefficient of infection, MR = moderately resistant, MS = moderately susceptible and S = susceptible. Means were separated at the least significant difference (LSD) at $P \leq 0.05$.

Data in Fig. (2) showed disease protection induced due to the foliar application of the tested materials against leaf rust of wheat (cv. Gemmieza-7) during the 2021/22 and 2022/23 growing seasons. High significant differences were recorded among all treatments and untreated control, while no significant differences were observed between both growing seasons. Nanochitosan was the most effective treatment, providing disease protection of 95% during both seasons, followed by folic acid (91.11%) during the first season and 95% during the second season comparable to the fungicide, being 94.44 and 93.75%, respectively. Nicotinic acid and ascorbic acid ranked second, where each showed protection against the disease by 91.11 and 90%, respectively. Chitosan provided disease protection by 82.22 and 80% during both seasons, respectively.

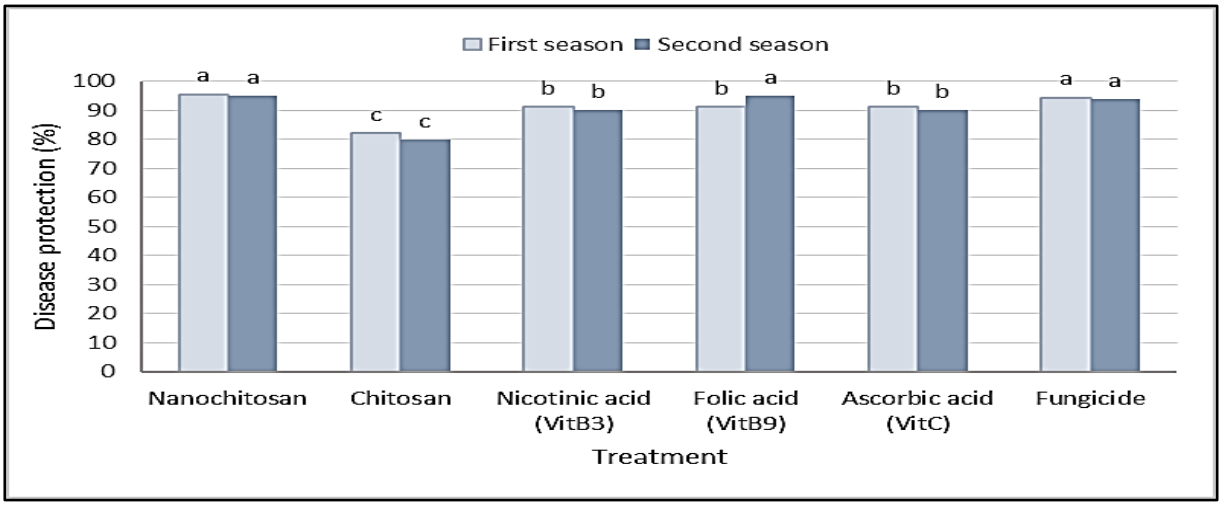


**Fig. (2): Disease protection induced by foliar application of nanochitosan, chitosan and vitamins (B3, B9and C) against leaf rust of wheat (cv. Gemmieza-7) under field conditions compared to the fungicide propiconazole 25% during 2021/22 and 2022/23 growing seasons.**

**Effect of nanochitosan, chitosan and vitamins on grain yield of wheat:**

Data shown in Table (2) indicated that all tested materials significantly increased the grain yield components of leaf rust infected-wheat (Gemmieza-7), *i.e.,* the spike weight (g), the 1000-kernel weight (g), and the volume weight(g), in comparison to the untreated control during both growing seasons. High significant differences were recorded among the treatments, except for the volume weights of the interaction between the treatments and seasons, which were not significant (ns). Nanochitosan gave the highest grain yield components during both growing seasons, followed by folic acid and nicotinic acid. Ascorbic acid and chitosan recorded the minimum significant increase in grain yield components during both growing seasons. In the first season, the best treatment nanochitosan recorded a spike weight of 4.44 g, a 1000-kernel weight of 62.87 g, and a volume weight of 722.13 g. Folic acid ranked second (4.33, 61.92 and 716.22 g), followed by nicotinic acid (4.18, 60.72 and 709.16 g), respectively. Ascorbic acid (3.65, 50.80 and 706.14 g), and chitosan (3.50, 47.28 and 705.84 g) recorded the minimum significant increase in grain yield components compared to fungicide (4.00 g, 60.88 g, 709.40 g) and untreated control (3.05, 39.68 and 639.80 g). In the second season, nanochitosan occupied the best with 4.81 g 63.56 g, and 724.28 g, followed by folic acid (4.45, 63.56 and 718.18 g) and nicotinic acid (4.34, 61.28 and 711.25 g), respectively. Ascorbic acid (3.81, 51.56 and 707.10 g), and chitosan (3.50, 47.28 and 705.84 g) ranked last with significant differences compared to fungicide (4.08, 61.28 and 711.50 g) and untreated control (3.05, 39.68 and 639.80 g).

**Table (2): Effect of foliar application of nanochitosan, chitosan and vitamins (B3, B9 and C) on grain yield components of leaf rust infected-wheat (cv. Gemmieza-7) under field conditions during 2021/22 and 2022/23 growing seasons.**

| Treatment | Spike weight(g) | | Mean | 1000-kernel weight(g) | | Mean | Volume weight(g/L) | | Mean |
|---|---|---|---|---|---|---|---|---|---|
| | 1st season | 2nd season | | 1st season | 2nd season | | 1st season | 2nd season | |
| Nanochitosan | 4.44 | 4.81 | 4.62 | 62.87 | 63.56 | 63.21 | 722.13 | 724.28 | 732.20 |
| Chitosan | 3.25 | 3.50 | 3.37 | 46.66 | 47.28 | 46.97 | 702.96 | 705.84 | 704.40 |
| Nicotinic acid (Vit. B3) | 4.18 | 4.34 | 4.26 | 60.72 | 61.28 | 61.00 | 709.16 | 711.25 | 710.37 |
| Folic acid (Vit. B9) | 4.33 | 4.45 | 3.39 | 61.92 | 63.56 | 62.74 | 716.22 | 718.18 | 717.20 |
| Ascorbic acid (Vit. C) | 3.65 | 3.81 | 3.73 | 50.80 | 51.56 | 51.18 | 706.14 | 707.10 | 706.62 |
| Fungicide (propiconazole) | 4.00 | 4.08 | 4.04 | 60.88 | 61.28 | 61.08 | 709.40 | 711.50 | 710.45 |
| Control (untreated) | 2.90 | 305 | 2.97 | 35.54 | 39.68 | 37.61 | 628.46 | 639.80 | 634.13 |
| LSD at 0.05 for means | | | | | | | | | |
| Treatments (T) | | | 0.07 | | | 0.85 | | | 6.81 |
| Seasons (S) | | | 0.04 | | | 0.46 | | | 3.64 |
| T × S | | | 0.09 | | | 1.21 | | | ns |

Means were separated at the least significant difference (LSD) at $P \leq 0.05$.

**Metabolic analysis:**

**Antioxidant enzyme activity:**

Antioxidant enzyme activities of peroxidase (POX) and catalase (CAT) were significantly increased in leaf rust-infected wheat leaves at the first and third-day post inoculation (dpi) after spraying with all treatments, *i.e.*, nanochitosan, chitosan and vitamins (B3, B9 and C) compared to untreated control, while they declined at the 7th dpi (Figs. 3 and 4). The highest enzyme activity of POX was observed with the application of nanochitosan, being 0.029, 0.065 and 0.025 µmol $min^{-1}$ $g^{-1}$ fresh weight (FW) at all the periods of study *i.e.*, 1, 3, and 7 dpi, respectively (Fig. 3). Folic acid (Vit. B9) came in the second order, being 0.026, 0.055 and 0.025 µmol $min^{-1}g^{-1}$ FW (Fig. 3). The highest enzyme activity of CAT was observed with nanochitosan treatment at 1 and 3 dpi of 45.0- and 57.0-mM $min^{-1}$ $g^{-1}$ FW, respectively, followed by nicotinic acid (Vit. B3) at 1 dpi (26.5 mM $min^{-1}$ $g^{-1}$ FW) and folic acid (Vit. B9) at 3 dpi (33.5 mM $min^{-1}$ $g^{-1}$ FW) (Fig. 4). Folic acid had the highest enzyme activity of CAT at 7 dpi (29.0 mM $min^{-1}$ $g^{-1}$ FW) (Fig. 4). Ascorbic acid (Vit. C) had the minimum efficacy for increasing the activity of POX, followed by chitosan (Fig. 3), while chitosan had the minimum efficacy in enhancing activity CAT, followed by ascorbic acid with the exception at 1 dpi in fungicide (Fig.4)

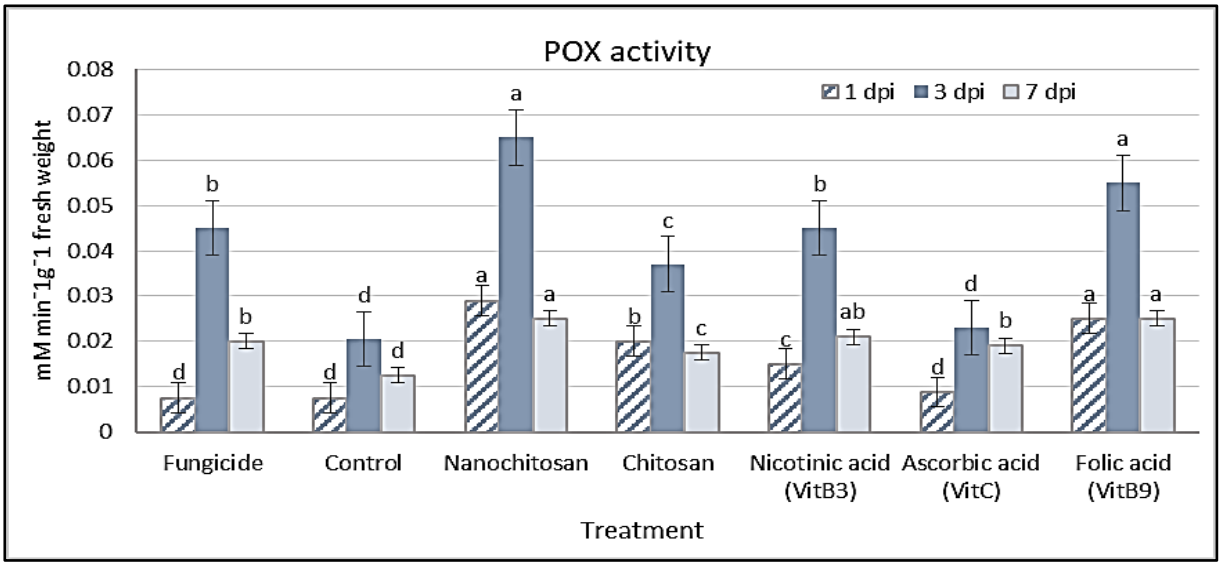


**Fig. (3): Antioxidant enzyme activity of peroxidase (POX) in leaf rust infected-wheat leaves (cv. Gemmeiza-7) treated with nanochitosan, chitosan and vitamins (B3, B9, C) as compared to the fungicide propiconazole 25% and untreated control.**

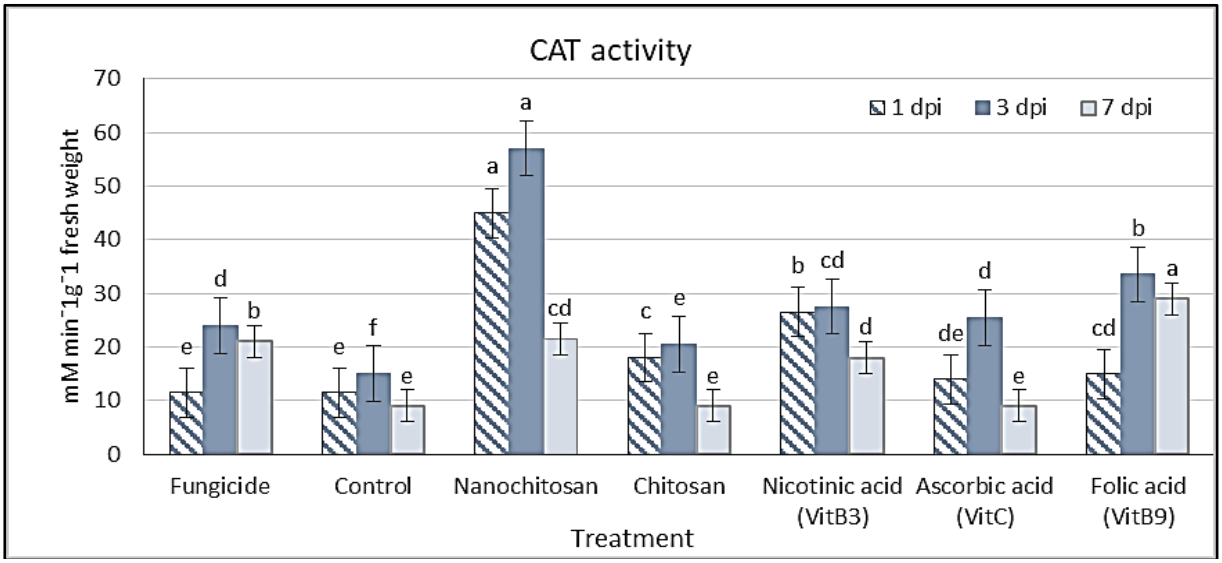


**Fig. (4): Antioxidant enzyme activity of catalase (CAT) in leaf rust infected-wheat leaves (cv. Gemmeiza-7) treated with nanochitosan, chitosan and vitamins (B3, B9 and C) as compared to the fungicide propiconazole 25% and untreated control.**

**Chlorophyll content:**

The total chlorophyll content (a + b) was significantly increased gradually in leaf rust-infected wheat leaves up to 7 dpi after spraying with the tested treatments *i.e.,* nanochitosan, chitosan and vitamins (B3, B9 and C) compared to the untreated control, which exhibited a noticeable decrease after the same period (Fig. 5). The highest concentration of total chlorophyll was recorded with nanochitosan treatment all over the periods of 1, 3, 7 dpi, being 3.99, 4.95, 4.99 mg $g^{-1}$, respectively, followed by folic acid, being 3.69, 3.88, 4.85 mg $g^{-1}$ and nicotinic acid (3.39, 4.63, 4.84 mg $g^{-1}$), which were comparable to the fungicide propiconazole 25% (3.34, 4.84, 4.89 mg $g^{-1}$). The least concentration of total chlorophyll was recorded with untreated control (2.63, 2.63, 3.25 mg $g^{-1}$).

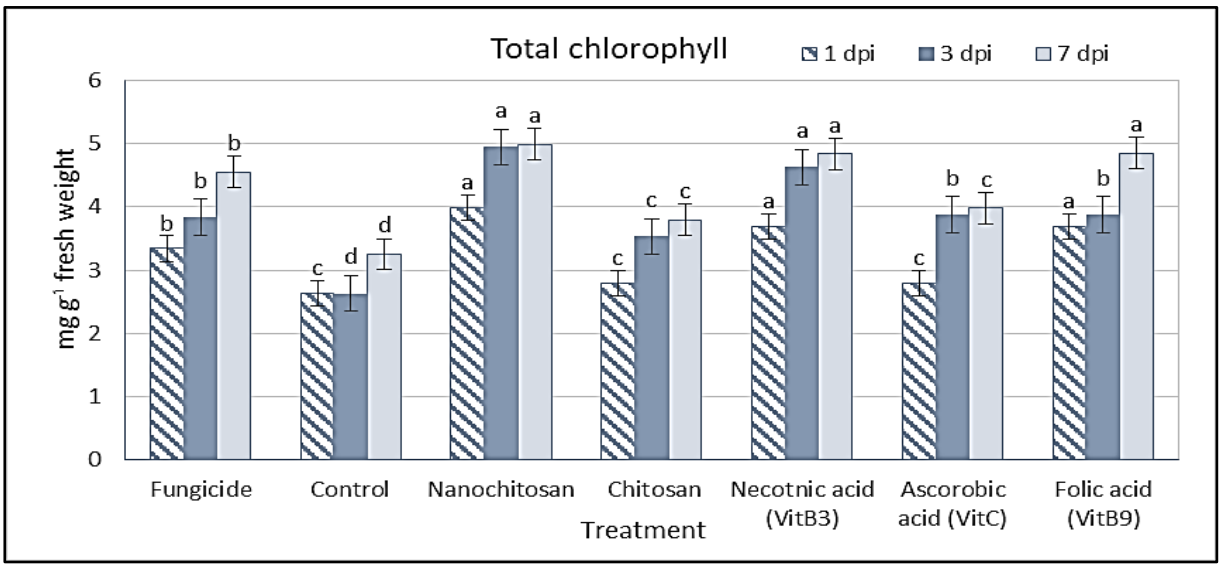


**Fig. (5): Concentration of total chlorophyll content in leaf rust infected-wheat leaves (cv. Gemmeiza-7) treated with nanochitosan, chitosan and vitamins (B3, B9 and C) as compared to the fungicide propiconazole 25% and untreated control.**

**Phenol content:**

The total phenol content was significantly increased gradually in leaf rust-infected wheat leaves up to 7 dpi after spraying with the tested treatments compared to untreated control (Fig. 6). The highest concentration of phenol was recorded with nanochitosan all over periods 1, 3 and 7 dpi, being 22.86, 35.06 and 65.72 mg $g^{-1}$, followed by folic acid, being 20, 33.68, 57.08 mg $g^{-1,}$ respectively compared to untreated control, being 9.46, 12.46, 25.95 mg $g^{-1,}$ respectively.

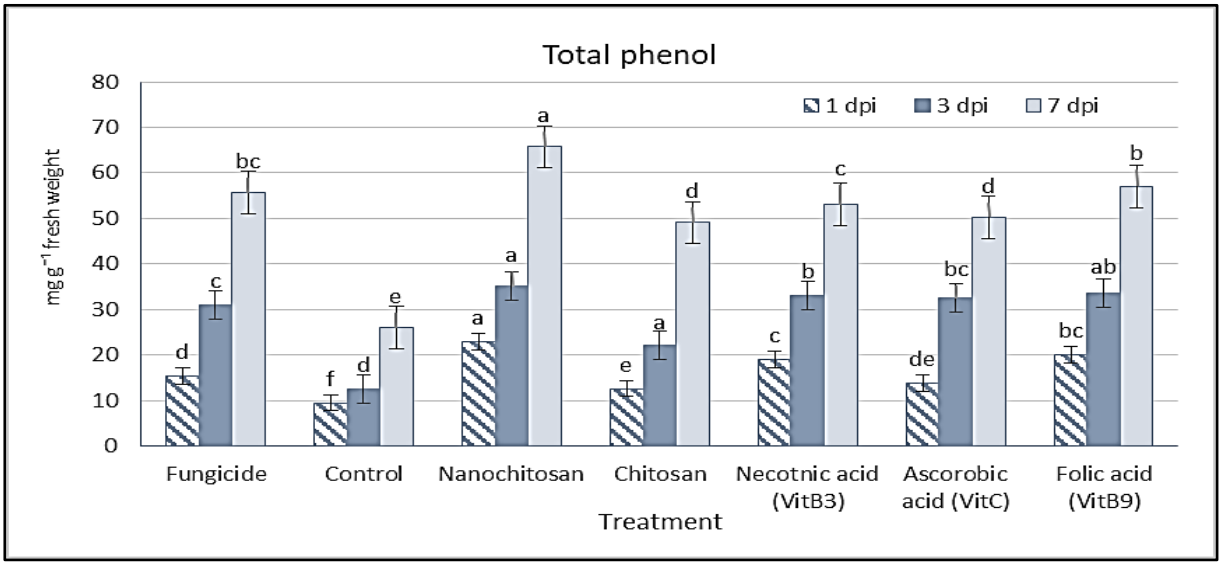


**Fig. (6): Concentration of total phenol content in leaf rust infected-wheat leaves (cv. Gemmeiza-7) treated with nanochitosan, chitosan and vitamins (B3, B9 and C) as compared to the fungicide propiconazole 25%.**

## DISCUSSION

Induced resistance (IR) is eco-friendly and offers sustainable protection against a variety of plant diseases (Durrant and Dong, 2004). The obtained data indicated that the application of nanochitosan, chitosan and vitamins (B3, B9 and C) before the infection by wheat leaf rust significantly reduced the disease under field conditions. These treatments offered good levels of protection reaching 95.0% by application of nanochitosan and folic acid (Vit. B9), which were comparable to the fungicide propiconazole 25% (93.75%). All the tested treatments elicited resistance to leaf rust and increased grain yield components. The plant responses were changed from susceptible (S) in untreated control and fungicide to moderately resistant (MR) by application of nanochitosan and folic acid (Vit. B9). Chitosan nanoparticles reduce spore germination and increase latency and periods of incubation meanwhile, decrease the type of infection, size, and pustules number compared to the untreated control (Elsharkawy *et al*., 2022). Chitosan nanoparticles have a potent antimicrobial effect due to their ability to bind microbial proteins and produce cell membrane permeability and disintegration (Juven *et al*., 1994). In the current study, chitosan nanoparticles were characterized using TEM. The micrograph displays chitosan nanoparticles with a variety of shapes, most of which are spherical and a few of which are sporadically oval with a homogenous population (Balaji *et al*., 2009). Nanoparticle size distributions in colloids ranged from 48.77 to 135 nm. Most of them had a diameter of about 88 nm for all colloidal solutions. Whereas just 12% of all liquids have particles larger than 100 nm. The poly disparity index (PDI), however, showed 0.87. Chitosan nanoparticles' size distribution has been demonstrated to range between 90 and 164 nm (Deng *et al*., 2006 and Ghadi *et al*., 2014).

Chitosan effects on hyphal development of plant pathogens (Xu *et al*., 2007). Resistance to *Botrytis cinerea* can be induced using chitosan (Al-Juboory *et al*., 2021). Chitosan is a widespread natural polymer with binary performance. First, it inhibits sporulation, spore germination, viability, and cell revisions of the pathogen, and stimulates diverse responses of plant defense. Second, during plant-pathogen contact, it elicits the plant host defense responses by inducing various biochemical reactions. Chitosan is a chitin derivative and is prepared by the deacetylation of chitin to varying degrees (Hassan and Chang, 2017). Chitosan and chitin fragments are known to elicit a host response to fungal infections due to phytoalexin accumulation lignin synthesis, pathogen-related proteins, proteinase inhibitors and formation of callose (El Hadrami *et al*., 2010). In the current study, chitosan also significantly reduced leaf rust infection providing a disease protection of 80% and increasing grain yield components. Folic acid (Vit. C), nicotinic acid (Vit. B3) and ascorbic acid (Vit. B9) have a depressant effect on both the growth and sporulation of leaf spot pathogen *Alternaria alternata* of beet (*Beta vulgaris*) (Singh *et al*., 1980). Different vitamins, including niacin (nicotinic acid) and folic acid, reduced downy mildew disease pearl millet and offered high protection against the disease (Pushpalatha *et al*., 2007). Application of vitamins like riboflavin provides effective protection from *Perenospora parasitica* of *A. thaliana* (Dong and Beer, 2000).

Induced resistance (IR) is characterized by an increase in the synthesized compounds in the host that can inhibit the development of the pathogen, due to the enhancement of the activity of antioxidant enzymes. Additionally, this causes a biochemical rise in phenol and chlorophyll over three times, and systemic resistance, up to the time samples were collected (Agrios, 2005). Our findings demonstrated a significant increase in the activities of POX and CAT enzymes in leaf rust-infected wheat leaves treated with all treatments. The maximum increase in enzyme activities of POX and CAT was recorded with nanochitosan treatment and this result is in agree with results obtained by Elsharkawy *et al*. (2022). In non-stressful circumstances, antioxidants both enzymatic and non-enzymatic effectively eliminate reactive oxygen species (ROS). However, under stress, ROS and antioxidant enzyme synthesis may be impacted. Recently, it has been demonstrated that ROS can function as immune defense stimulants and signaling molecules in biosystems (Yang and Gaojian, 2020). According to several studies, chitosan and its derivatives work through a variety of mechanisms, including preventing pathogen growth and inducing a host defense response (Xing *et al*., 2015). It has been demonstrated that chitosan increases the activities of defense enzymes in *Pinus koraiensis* seedlings 2 days after application (Liu *et al*., 2014). Chitosan controls a variety of plants defense genes, including activating defense mechanisms (Pichyangkura and Chadchawan, 2015). Infected

pearl millet plants with downy mildew have higher levels of defense enzymes after exposure to chitosan nanoparticles (Siddaiah *et al*., 2018). Wheat PR1-PR5 and PR10 defense genes against leaf rust could be activated by chitosan nanoparticles (Elsharkawy *et al*., 2022). The induction of systemic resistance due to chitosan nanoparticles was correlated with the transcriptional levels of pathogen-related protein-1 phenylalanine ammonium lyase and peroxidase (Elsharkawy *et al*., 2022). The current investigation found a correlation between the level of disease protection and the elevated peroxidase and catalase activity.

Menadione sodium bisulfite (Vit. K3), para-aminobenzoic acid (Vit. Bx), Thiamine (vitamin B1), riboflavin (vitamin B2) and folic acid (Vit. B9) are vitamins that offer effective protection against a variety of diseases by modulating particular host-defense characteristics. Ascorbic acid and tocopherols, two additional vitamins, are components of the molecular pathways behind induced resistance. Vitamins function as cofactors of the flavoprotein enzymes, some of which catalyse lipid peroxidation, a key step in the production of reactive oxygen intermediates (ROIs), which act as a signaling network in plant immunological responses (Fischer and Bacher, 2006). An innovative signaling route that results in systemic resistance is activated by riboflavin. Riboflavin may have started the resistance signal transduction since it boosted peroxidation and antioxidant defense enzymes in *A. thaliana* against *Peronospora parasitica*. This, in turn, activated PR genes (Dong and Beer, 2000).

Plants can synthesize phenols, phenolic acids, flavonoids, flavones, flavanols, quinones, tannins and coumarins, which are fragrant secondary metabolites (Cowan, 1999). Carvacrol, eugenol, and thymol are examples of substances with phenolic compounds that are very effective against pathogens infections. Ascorbic, humic and nicotinic acids increase total carbohydrates and oil percentage, total flavonoids, total alkaloids and protein percentage in fenugreek (Mohamed *et al*., 2015). Ascorbic acid (Vit. C) and tocopherols (Vit. E) are modulated by elicitors of disease resistance in plants (Wolucka *et al*., 2005 and Khan *et al*., 2012). For the biosynthesis of some amino acids, folic acid (vitamin B9) is essential in all organisms (Hanson and Gregory, 2011).

As a result of these other properties that increase host plant defenses, interest in utilizing integrated disease management to lessen the destructive effects of diseases on crop production. Nanochitosan, chitosan and vitamins, B3 (nicotinic acid), B9 (folic acid) and C (ascorbic acid) will gain popularity as a plant protectant for reaching the objective of sustainable agriculture.

## CONCLUSION

Prior infection application of nanochitosan, chitosan and vitamins, B3 (nicotinic acid), B9 (folic acid) and C (ascorbic acid) as resistance inducers offered good levels of disease protection of wheat leaf rust under field conditions. They decreased disease infection and increased grain yield components. Treatment with nanochitosan was the most effective. Metabolic aspects involved in induced resistance in wheat resulted in increasing antioxidant enzymes activities (POX and CAT), chlorophyll and phenol contents. The materials used seem promising to achieve sustainable agricultural goals, especially when applying nanotechnology. To apply this technique to plant diseases, it is worth paying attention to the method and formulation.

## CONFLICTS OF INTEREST

The author(s) declare no conflict of interest.

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