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A Quick PCR detection of *Ustilago tritici* for early control of loose smut in wheat using TiO_2 and ZnO_2 nanoparticles

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ABSTRACT

Loose smut disease, caused by *Ustilago tritici*, is a significant disease of wheat. Embryo examination and PCR detection were used to detect *Ustillago tritici* in seeds. The disease was controlled using TiO_2 and ZnO_2 nanoparticles. Out of 12 wheat cultivars, three—Sakha-61, Sakha-93, and Gemmeiza-11—had highly significant percentages of embryos infected with *U. tritici*, up to 65%, with infection severity values up to 15.3%. Sakha-95, Sids-14, Misr-1, Misr-2, and Misr-3 Cultivars exhibited no infection in the lab; however, in the field, the infection rate reached up to 30%. To enhance the detection of infected seeds, a modern technique using PCR biotechnology has been developed. This method has revealed that embryos in cultivars previously considered uninfected based on laboratory tests are actually infected. From another perspective, TiO_2 and ZnO_2 nanoparticles reduced the mycelial radial growth of *U. tritici*, decreasing the infection from 87% to less than 10% compared to the control, and performed comparably to Hattrick fungicide under greenhouse conditions. The treatment increased the expression profile of TaPR5 compared to untreated plants. The findings of this study provide compelling evidence of the high efficacy of these nanoparticles in promoting plant development and suppressing disease. These potent nanoparticles could replace traditional methods of plant growth enhancement and disease management.

Keywords: Breed wheat, *Ustilago tritici*, embryos examination, molecular markers, nanoparticles.

INTRODUCTION

One of the most harmful diseases that affect wheat cultivars worldwide, is known as wheat smut which caused by *Ustilago segetum* var. *tritici*. In some areas, this disease is a significant obstacle to sustaining productivity and quality, and when vulnerable cultivars are grown, the impact can be very severe. Smuts was one of the primary worries of farmers in the majority of wheat-growing countries up until the 20th century (Agrios, 2005). Although they can transfer short distances, infected seeds are the primary means of spreading loose smut (Nielsen and Thomas, 1996). The environment significantly influences the growth and spread of teliospores, with optimal conditions between 22-27 °C and 60-90% relative humidity. Yield reduction is similar to smutted heads, as smutted spikes result in grain loss (Gad *et al*., 2019). The loose smut disease is internally seed-borne, meaning that the spores stay in the seed embryo. When conditions are favorable, it then damages the new plant that was grown from the original infected seed. The sickness is typically observed at higher altitudes, and cool, wet weather is conducive to the disease (Sehsah, 2019). When farmers reuse their wheat seeds, contaminated seeds are the only source of perpetuation disease, and loose smut reduces yields by up to 5-7% (Ramdani *et al*., 2004). Present agriculture needs to introduce wheat yield and decrease the risk of diverse biotic and environmental challenges. Nanotechnology is gaining a lot of attention among these technological advancements due to its wide variety of applications in the creation of nano-pesticides, nano-sensors, and smart delivery systems for controlled and sustained release of agrochemicals in agriculture. The productivity, yield and agronomic characteristics of plants have so far been proven to be both positively and negatively impacted by nanoproducts, including, e.g., changes to the nutrient content of food crops. The success of nanomaterials to be applied depends on the processes and routes of nanoparticles (NPs) penetration, absorption, and migration in addition to application methods to reduce toxicity and adverse effects in many plants (Rai *et al*., 2012; Kashyap *et al*., 2020; Prem *et al*., 2020; Derbalah *et al*., 2022; Elsharkawy *et al*., 2022a; Elsharkawy *et al*., 2022b; Kamel *et al*., 2022).

In recent years, pathogenic fungi have become increasingly resistant to some fungicides and commercial antimicrobial treatments. This prompted researchers to look for novel approaches to treat microbial and fungi-related infections (Abd El-Rahman *et al*., 2021; Elsharkawy *et al*., 2022b). As application-related aspects of nanotechnology develop, using NPs to manage plant diseases is a novel approach that could

become very effective in the future. Nanoparticles may control the pathogen, just like chemical pesticides do. In addition, NPs can transport pheromones, substances that cause systemic acquired resistance (SAR), and inhibitors of polyamine synthesis (Khan *et al*., 2014). Titanium dioxide nanoparticles (TiO_2NPs), one of the most important materials in nanotechnology, stimulated the growth of oilseed rape roots. These plants were also protected against seed and soil-borne diseases like *Alternaria brassicae* infection and had adhesive effects on bacteria *Bacillus amyloliquefaciens* (Palmqvist *et al*., 2015).

When interacting with bacteria and plants, TiO_2NPs can act as protective encasing agents that strengthen the adhesive force (Webster *et al*., 2008; Chowdhury *et al*., 2012). According to results obtained by Ruffolo *et al*. (2010) and Song *et al*. (2014), zinc titanium oxide ($ZnTiO_3$) and Zn hydroxide carbonate nanoparticles (NPs) demonstrated greater ability to inhibit the growth of Aspergillus niger and fungal activity against cotton Verticillium, Rhizopus, and Mucorales than zinc oxide nanoparticles (ZnO NPs). Abd-Elsalam (2013) and Mahendra et al. (2012) highlighted the importance of using grower-friendly NPs in agriculture to protect crops and prevent losses due to plant infections and diseases. To develop a more efficient and secure alternative to fungicides, it was important to test nanoparticles on various diseases, including loose smut in wheat. The study aimed to rapidly detect *U. tritici* in healthy seeds using a PCR-based method. In addition to investigate how titanium dioxide (TiO_2) and zinc peroxide (ZnO_2) nanoparticles control loose smut in wheat.

MATERIAL AND METHODS

Survey of seed-borne pathogens of wheat seeds:

A survey of seed-borne pathogens in wheat seeds was carried out under laboratory conditions. Seed samples of twelve wheat cultivars, namely, Sakha-61, Sakha-93, Sakha-94, Sakha-95, Gemmeiza-11, Gemmeiza-12, Sids-12, Sids-13, Sids-14, Misr-1, Misr-2, and Misr-3, were collected from fields and storehouses in different locations of Kafr Elsheikh governorate to isolate its seed-borne fungi according to the methods adopted by a study as shown in Fig. (1). The sample of each cultivar was prepared by mixing the individual samples and preserving them in cloth bags in laboratory conditions at room temperature during the studies. Seed-borne fungi of the studied cereal crops were isolated using agar plate methods (PDA) as recommended by the International Seed Testing Association (Agarwal, 1976; ISTA, 1996). The fungi were identified based on their morphological and reproductive characteristics (Sharifnabi *et al*., 2000).

Embryo count method and microscopic examination of infected seeds:

The presence of *Ustilago tritici* in the embryonic seeds was examined microscopically in this study. 100 g of each wheat seed sample was socked in 1000 ml of fresh 5% sodium hydroxide (NaOH) solution containing 0.01–0.001 percent trypan blue overnight at 20 °C. After soaking, each sample was transferred to a suitable container and washed with warm water to segregate the embryos that appeared through raptured pericarps. The Fenwick Cane was fitted with a basal hot water inlet. The flow of water helped to separate the embryos and carried them to the top of the cane. Further instigation could be provided by stirring. It was observed that embryos flowed over the lip of the Fenwick Cane and were caught in a sieve of 1 mm mesh. Additional sieves of larger mesh could be used above to collect pieces of endosperm and chaff. Once one of the embryo samples had been obtained, chaff and other debris could be separated from the embryos in a funnel containing a mixture of lactic acid, glycerol, and water (1:2:1). The embryos floated, and the chaff sank and could be removed through a rubber tube with a stop clip. Repeating the process several times until a reasonably clean sample was obtained. Finally, the embryos were cleared with lactic acid and glycerol (1:2), which were maintained at the boiling point for examination after cooling. The fungus was identified, according to Mathur and Cunfer (1993).

PCR detection of infected seeds:

A modern method was developed using PCR biotechnology and the use of nanoparticles of both titanium dioxide and zinc peroxide in the process of extracting DNA from seeds to increase the chance of precipitation to improve the detection of embryos infected with PCR (Dellaporta *et al*., 1983). The sequence, size, and specificity of primers were used for the fungi's DNA amplification as follows:

Uh1 Uh4	5'- CGCACCTGTCCAACTAAAC 3' 5'- GAGGTTGAGATGGGTAGGA 3'	574-bp	Willits and Sherwood, 1999

The program of PCR consisted of an initial denaturation of 3 min. at 94 °C, followed by 40 cycles of 1 min. of denaturation at 94 °C, 1 min. of annealing at 57 °C, and 1 min. of extension at 72 °C. The final extension was 7 minutes at 72 °C. The amplification DNA was electrophoresed in 1% agarose gel for *U. tritici* in 1XTBE buffer at 120V for 1 hour, stained with ethidium bromide (0.5/ml), and photographed using a UV lamp in gel documentation (Bio-Rad, Gel Doc XR system 170-8170). The molecular weight of the PCR products was determined by comparison with the DNA marker weights VC 100 bp plus DNA ladder (Vivantis) and VC 1 kb plus DNA ladder (Vivantis).

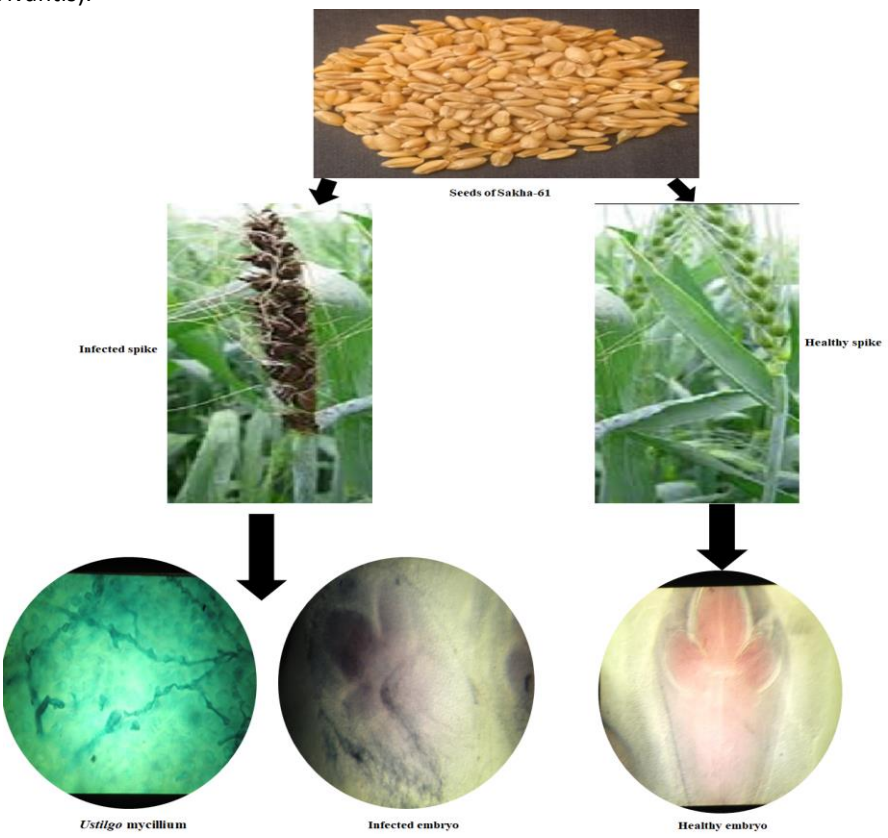

Fig. 1. Symptoms of loose smut on spike as well as healthy and infected embryo grains.

Evaluation of certain wheat cultivars for loose smut disease under the greenhouse:

The response of twelve wheat cultivars such as Sakha-61, Sakha-93, Sakha-94, Sakha-95, Gemmeiza-11, Gemmeiza-12, Sids-12, Sids-13, Sids-14, Misr-1, Misr-2, and Misr-3 was detected to the artificial inoculation of a loose smut in a plastic greenhouse. The wheat plants of the aforementioned CVs. were artificially inoculated with the pathogen at the flowering stage in the years 2021 and 2022. The seeds from individual inoculated panicles were harvested and sown one more time during the next season. Then the percent of infection by *U. tritici* in the panicles of the grown plants was calculated.

***In vitro* antifungal activities of two nanoparticles on linear growth of *U. tritici* under laboratory condition:**

Two TiO_2 and ZnO_2 nanoparticles (Nanotech Company limited, Cairo, Egypt) and Hattric fungicide (Tebuconazole 6% FS) were used in this study to determine their effect on the growth of the pathogen. The colloidal particle solution was diluted to different concentrations, such as 25, 50, and 100 ppm, compared to Hattric fungicide, which was used as the recommended dose (0.7 ml/1 kg seeds). Each of the tested particles was incorporated at the determined concentration into the PDA medium at 45 °C. The media were thoroughly mixed, poured into Petri dishes, and left to solidify at room temperature. Plates were inoculated at the center with 6 mm-diameter equal discs taken from a 20-day-old culture of *U. tritici* grown on PDA medium at 24 °C. Four replicates were maintained for each treatment. Linear growth was recorded daily until the PDA medium was dried (20 days), indicating the growth of the fungus (Aurangzeb *et al*., 2003; Kim *et al*., 2009; Min *et al*., 2009). The inhibition percent was calculated using the formula of (Aurangzeb *et al*., 2003):

$$R = \frac{C - T}{C} \times 100$$

R = Percent of inhibition of the fungal growth.
C = Linear growth of untreated plates.
T = Linear growth of treated plates with the used treatments.

Effect of the two nanoparticles against loose smut disease under greenhouse conditions:

The effect of TiO_2 and ZnO_2 (100 ppm) on loose smut was studied on the Sakha-61 cultivar compared to a Hattric fungicide (0.7 ml/kg) under greenhouse conditions. The used natural materials were tested by seed-soaking for three hours (Sluosarenko *et al*., 2008; Ghosh *et al*., 2005). Then it was air-dried for one hour and sown as described above. The total number of smutted heads was recorded, and the percent of loose smut infections was calculated.

Transcriptional levels of genes related to defense:

For each treatment, 100 mg of wheat leaves were extracted for total RNA extraction. The RNA was purified using the Thermo Scientific Fermentas kit #K0731 (Elsharkawy *et al*., 2022b). Once the concentration was determined, the integrity and purity of the RNA were determined using agarose gel electrophoresis and a Nano SPECTROstar. The kits (Thermo Scientific, Fermentas, #EP0451) were used for reverse transcription. The generated cDNA was utilised for qRT-PCR amplification using specified primers to detect TaPR5 expression patterns (/5CAAGCAGTGGTATCAACGCAGAG/3) and (/3GTGAAGCCACAGTTGTTCTTGATGTT/5). This gene's transcripts with program traits were amplified using a real-time cycler (Desmond *et al*., 2006). Three biological and technical replicates were used for each treatment. To calculate the relative expression levels, the technique proposed by Livak and Schmittgen (2001).

Statistical analysis:

The random complete block design (RCBD) with three replicates was utilized. Analysis of variance (ANOVA) was used to analysis the data using the statistical analysis software SPSS22.

RESULTS

Embryos count:

Microscopic analysis of the presence of the fungus *U. tritici* in seed embryos from 12 wheat cultivars, including Sakha-61, Sakha-93, Sakha-94, Sakha-95, Gemmeiza-11, Gemmeiza-12, Sids-12, Sids-13, Sids-14, Misr-1, Misr-2, and Misr-3 (Fig. 2), revealed that three of the wheat cultivars, Sakha-61, Sakha-93, and Gemmeiza-11, had highly significant percentages of embryos infected with *U. tritici* up to 65%. Sakha-95, Sids-14, Misr-1, Misr-2, and Misr-3 cultivars did not exhibit any infection in the lab, but when they were planted in the field, they displayed an infection rate of up to 30%, demonstrating the distinctions between the healthy and diseased forms. Grain embryos from both healthy and *U. tritici* infected grains, as well as ears, as shown in **Fig. (1).**

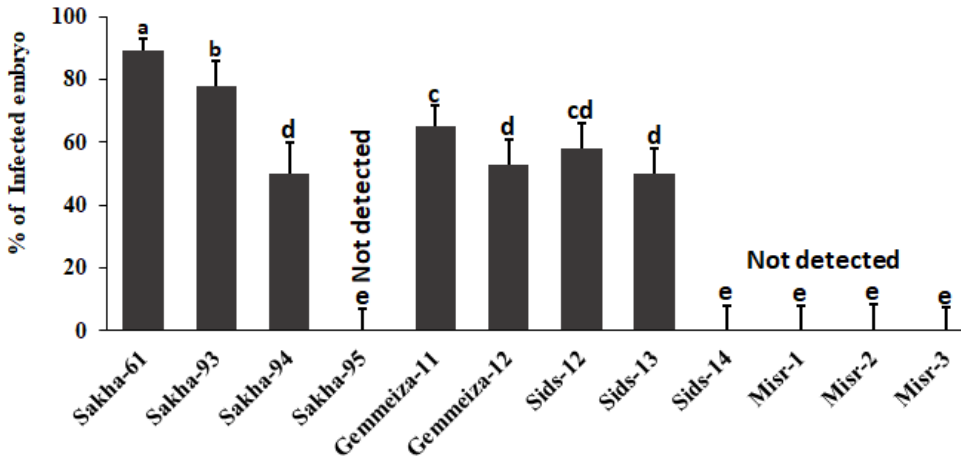

Fig. 2. Embryos count of twelve wheat cultivars for infection with *U. tritici* under laboratory conditions.

PCR detection of *Ustilago tritici* in seeds:

The differences between whole DNA extraction from dry wheat seeds of Sakha-95 by adding different analyzer buffers, such as Dellaporta, titanium dioxide and zinc peroxide NPs. The whole DNA extracted using Dellaporta buffer, when added to Tio_2NPs, gave the highest quantities of DNA in all buffers and more different samples. In the first season, DNA extracted by Dellaporta buffer + Tio_2NPs yielded 3114.1 ng/µl, followed by Dellaporta buffer + Zno_2NPs (2143.3 ng/µl) and Dellaporta only (1927.3 ng/µl). In the second season, DNA was extracted by Dell. Buffer with Tio_2NPs had the highest quantity (2897.1 ng/µl), followed by Dell. +Zno_2NPs (2291.3ng/µ) and Dell. only (1871.3 ng/µl). From a general perspective, the extraction by Dell Tio_2NPs had the highest yield of DNA, while Dell buffer was the lowest result. On the other hand, *Ustilago tritici*-specific primer pair Uh1/Uh4 was used to detect a 574 bp PCR product using a 1 kb ladder. Lanes 1 and 2 showed very

distinctive, strong, sharp bands of DNA template extracted from fungus pure culture and infected seeds using Dellaporta buffer, compared to Lane 3, which shows a weak band extracted by Tio_2NPs. Lane 4 showed no band when extracted by Zno_2NPs. Lane 5 showed no band as a negative control.

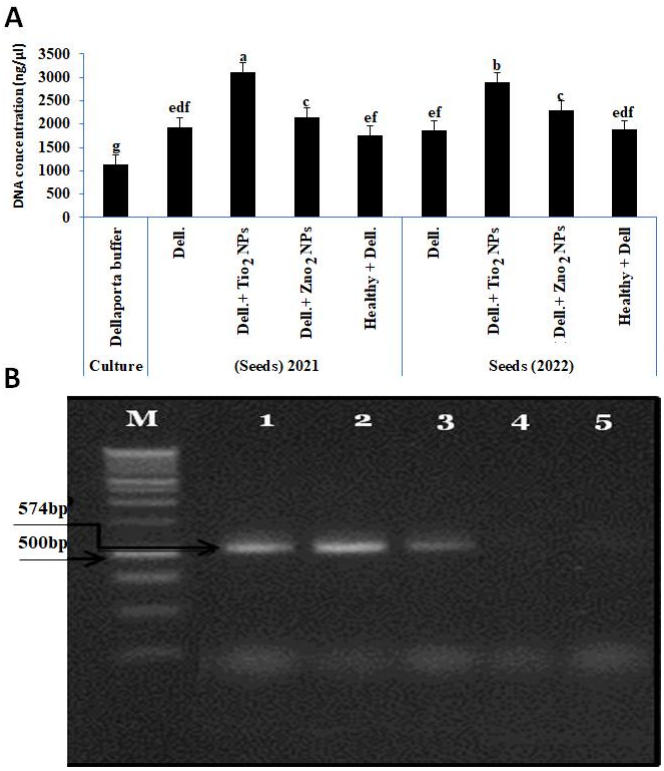

Fig. 3. (A) Purified DNA concentration (ng/µl) extracted from dry seeds of Sakha-95, and (B) gel electrophoresis of the detected *Ustilago tritici* in Sakha-95 using the specific primers pair Uh1/Uh4 (574pb). M: DNA marker, Lane 1: *U. tritici* DNA template from culture extracted by Dellaporta buffer, lane 2: DNA template from wheat dry seeds extracted by Dellaporta buffer, lane 3: DNA template from wheat dry seeds extracted by Dellaporta + Tio_2NPs, lane 4: DNA template from wheat dry seeds extracted by Dellaporta + Zno_2NPs and lane 5: DNA template from healthy seeds sample used as negative control.

Infection severity % of *U. tritici* under greenhouse conditions:

The twelve wheat cultivars, i.e., Sakha-61, Sakha-93, Sakha-94, Sakha-95, Gemmeiza-11, Gemmeiza-12, Sids-12, Sids-13, Sids-14, Misr-1, Misr-2, and Misr-3, were evaluated for the fungus *U. tritici* in two years, 2021 and 2022. Figure 4 shows that three wheat cultivars, Sakha-61, Sakha-93, and Gemmeiza-11, had high values of infection severity up to 15.3%, while Sakha-95, Sids-14, Misr-1, Misr-2, and Misr-3 had the lowest value of infection severity, less than 5.2%, in the tested two years.

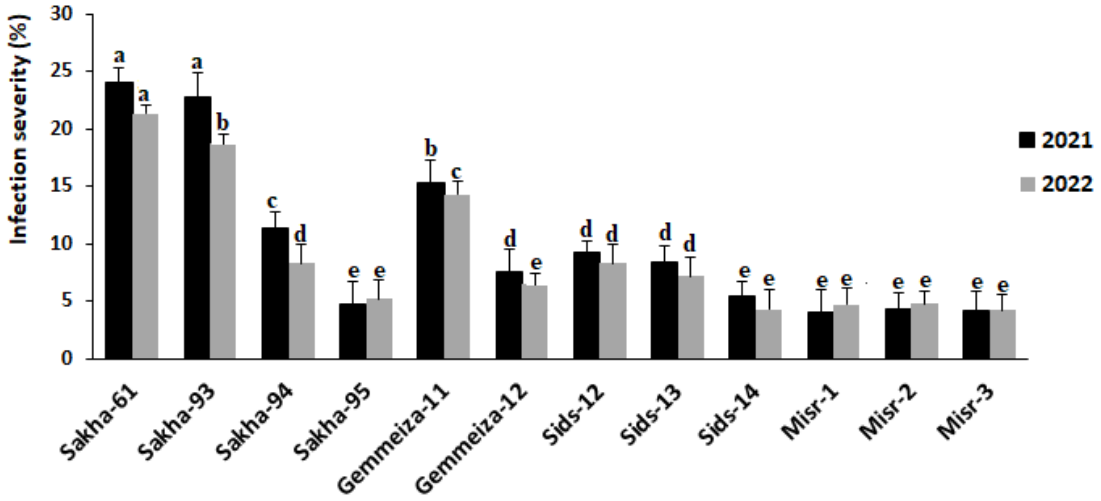

Fig. 4. Test of twelve wheat cultivars for infection with *U. tritici* under greenhouse conditions.

Effect of TiO_2NPs and Zno_2NPs on radial growth and growth inhibition of *U. tritici in vitro*:

Data in Table (1) revealed that the effects of TiO_2 and ZnO_2 nanoparticles in comparison to Hattric fungicide on the growth of *U. tritici* fungus were various at four different concentrations (10, 20, 50 and 100 µg/mL), where the most effective concentration for two nanoparticle treatments and fungicide was 100 g/mL. Due to TiO_2NPs treatment, the radial growth/mm of *U. tritici* fungus was high in low concentrations of TiO_2NPs and ranged from 0.53 to 1.40/mm; the growth inhibition% was more efficacious in high concentrations of 100 µg/mL and ranged from 67.44 to 87.67 % (Table 1 and Fig. 5B). The effect of Zno_2NPs on the radial growth/mm of *U. tritici* fungus (Fig. 5C) ranged from 0.68 to 1.20/mm, and the growth inhibition% was up to 84.18% at a high concentration of 100 µg/mL, compared to the positive control (Hattric) and the negative control (sterile distilled water). The radial growth per mm ranged from 0.50 to 0.98 mm (Fig. 5D) and 4.30 mm (Fig. 5A), respectively. The coefficient of determination (R^2) was measured in TiO_2 and ZnO_2 nanoparticles and the fungicide Hattric, where it was 0.8843, 0.8430, and 0.9835, respectively. The regression equation was generated for every treatment to predict the efficacy of various treatments.

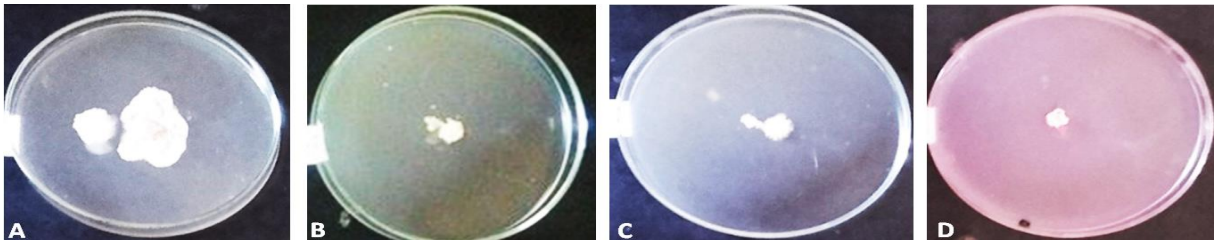

Fig. 5. The effect of tested treatments on the Radial growth/ mm of *U. tritici in vitro*. (A) control, (B) TiO_2 NPs, (C) ZnO_2 NPs and (D) Hattric.

Table 1. Radial growth, % growth inhibition, regression equation, and degree of correlation of the tested treatments against *U. tritici* in vitro.

Treatment	Concentration (µg/mL)	Radial growth (mm)	Growth inhibition %	Regression equation	R^2
TiO_2	10	1.40	67.44	y = -0.0094x + 1.3872	0.8843
	20	1.20	72.09		
	50	0.72	83.25		
	100	0.53	87.67		
ZnO_2	10	1.20	72.09	y = -0.0052x + 1.1531	0.8430
	20	1.00	76.74		
	50	0.79	81.62		
	100	0.68	84.18		
Hattric	10	0.98	77.20	y = -0.005x + 1.0068	0.9835
	20	0.87	79.76		
	50	0.77	82.09		
	100	0.50	88.37		
Control	0.0	4.30	0.00	-	-

Effect of TiO_2NPs and Zno_2NPs on infection severity (%) of *U. tritici*:

The two treatments, TiO_2 and ZnO_2 nanoparticles, were tested against the *U. tritici* infection. The treatments resulted in a reduction in infection severity (%) of less than 10% compared to the control and had nearly the same efficacy as the fungicide Hattric, which reached more than 80% in the tested two years of 2021 and 2022 (Figs. 6A and B).

Accumulation of TaPR5 transcripts in response to induction treatments:

The expression profile of TaPR5 in wheat leaves infected with *U. tritici* was determined using qRT-PCR analyses. The results revealed that relative expression was significantly increased in all treated wheat plants (Fig. 7). TaPR5 was significantly greater in the TiO_2NPs and ZnO_2NPs-treated plants compared to infected plants (untreated plants) (Fig. 7).

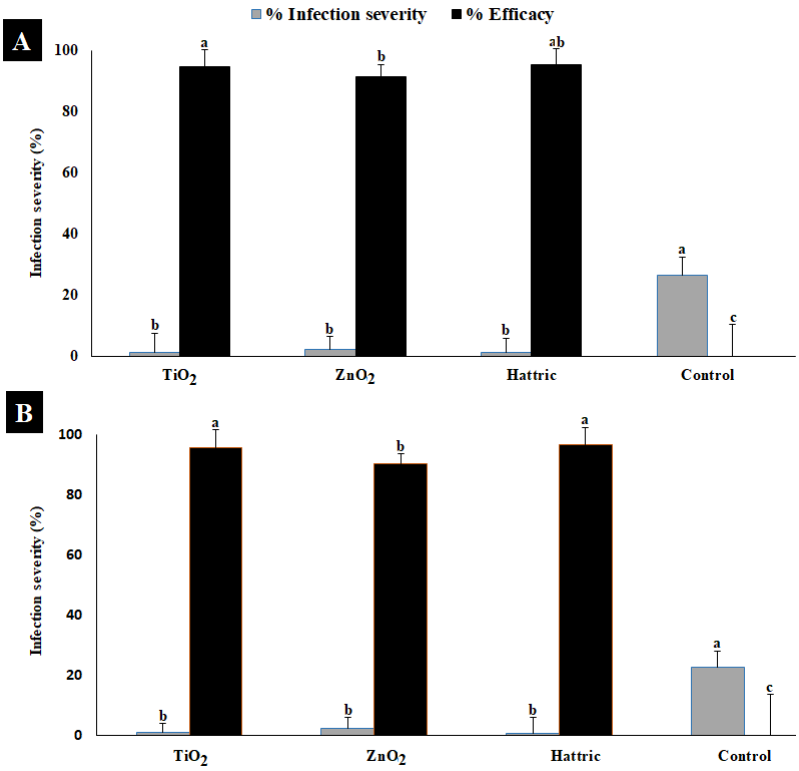

Fig. 6. Effect of two nanoparticles on infection severity (%) compared to Hattric fungicide, during 2021 (A) and 2022 (B) seasons.

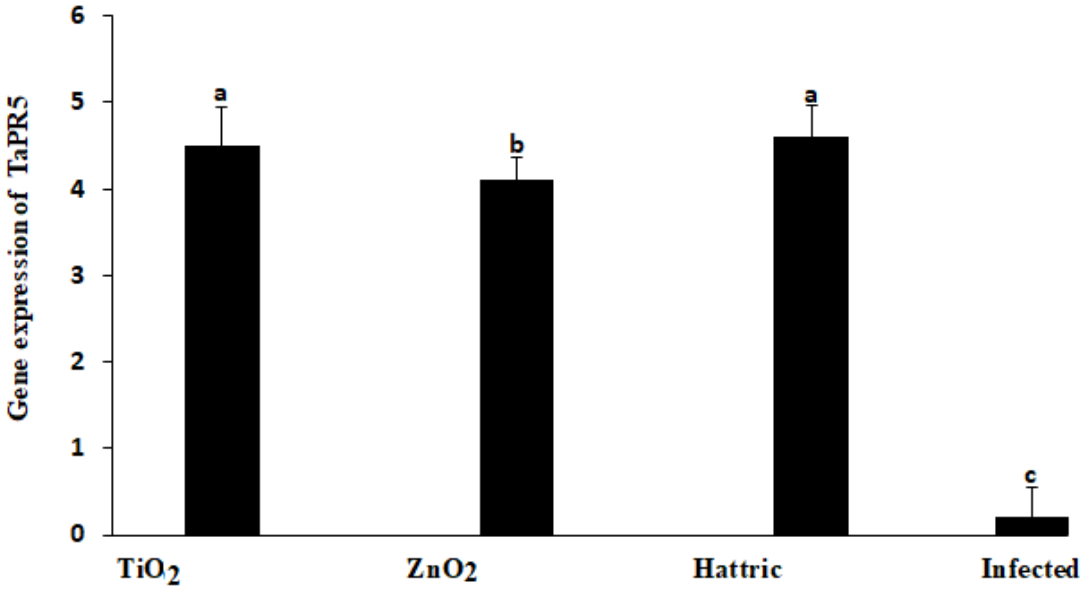

Fig. 7. Effect of TiO_2NPs and Zno_2NPs on the relative expression level of TaPR5 gene in infected wheat plants.

DISCUSSION

The present study aims to develop methodologies for seed health that would make it possible to diagnose and control fungal-borne diseases in a reliable and timely manner. *Ustilago tritici*, is a dangerous seed-borne fungus found in wheat. It's difficult to detect using traditional seed health tests, and even more difficult to treat. It can be difficult to test seeds for fungal infections. Aveling (2014) claims that according to many researchers, including Tsedaley (2015), contaminated seeds cannot show any signs, making visual diagnosis difficult. There are many ways to identify different seed-borne diseases; However, few requirements for appropriate seed tests have been met. Optimal seed health methods must be sensitive, targeted, rapid,

reliable and affordable. The embryo count method showed that three wheat cultivars, Sakha-61, Sakha-93, and Gemmeiza-11, had very high percentages of embryos infected with *U. tritici*. Sakha-95, Sids-14, Misr-1, Misr-2, and Misr-3 cultivars did not show any rates of embryonic infection. While, grown in the field, it showed an infection rate of up to 30%. Accordingly, Rewal and Jhooty (1982), who indicated that the embryo count method cannot be used to detect infection of less than 50% within wheat seeds. While, a modern method was developed using PCR biotechnology and the use of nanoparticles of both iron and silver in the process of extracting DNA from seeds to increase the chance of precipitation to improve the detection of embryos infected with PCR. Indeed, this method has proven the infection of embryos in cultivars in which the infection was not detected by examining the embryos in the laboratory. Therefore, it is preferable to rely on this method for rapid detection within 24 hours, which saves the state and farmers from economic losses as a result of pesticide treatment, as well as crop losses.

On the other hand, nanoparticles have been developed for agricultural use in recent years to improve physiological processes and are promoted as mineral enhancers, especially important micronutrients needed for host defense, as they act as resistance inducers and reduce the presence of harmful organisms (Elsharkawy *et al*., 2018, Elsharkawy *et al*., 2022a, Elsharkawy *et al*., 2022b). Some researchers, such as Lamsal (2011), Raskar & Laware (2014) and Servin *et al.* (2015) emphasize nanoparticles' potential use as antifungal agents, which could limit the transmission of various fungi and their infection. Additionally, Mahendra *et al*. (2012) and Abd-Elsalam (2013) emphasized that NPS needed to be simple to apply to guard against plant pathogen diseases. Several studies have been published and demonstrated how effective ZnO_2NPs and TiO_2NPs are at enchancing DNA yield in DNA extraction and controlling many diseases. So, they were used in this study to control loose smut disease.

In vitro, TiO_2 and ZnO_2 nanoparticles compared to Hattric fungicide were more effective in reducing the growth of *U. tritici* fungus at the best concentration of 100 g/mL. The coefficient of determination (R^2) was positive and strong between the effective two nanoparticles and radial growth (mm). Likewise, Aspergillus niger fungal colony growth has been inhibited on Petri dishes using $ZnTiO_3$ (Ruffolo *et al*., 2010). Similar growth inhibition appears to be seen in formulas produced from different polymers but using the same metal oxide. Furthermore, $ZnTiO_3$ limits fungal growth more than ZnO (Wang and Lin, 2008).

Under greenhouse conditions, TiO_2 and ZnO_2 nanoparticles reduced the infection severity (%) of *U. tritici* compared to the control. They achieved the highest efficacy of reducing the infection severity compared to Hattric fungicide during the two years under study. This result was supported by Farahat (2018), who found that TiNPs and ZnNPs reduced disease severity percentages of cercospora leaf spot (CLS) in sugar beet plants and enhanced TSS and sucrose contents. As a mechanism of resistance, NPs caused activation and high enzyme activity values of peroxidase up to 6 min and polyphenol oxidase up to 4 min, respectively, compared to the control. Also, TiNPs increase nitrate reductase, improve water absorption, stimulate antioxidant effects, speed up germination, and increase light absorption (Lu *et al*., 2002). Hamaza *et al*. (2013) reported that ZnNP suppressed the damping of charcoal rot diseases in sunflowers and controlled late wilt disease in maize. It increased yield because zinc oxide nanoparticles had the dual role of being an essential nutrient and a co-factor for nutrient-mobilizing enzymes. Selecting the proper concentration of nanoparticles is important for realizing higher benefits for a target agro-economic trait. In the same vein, Palmqvist *et al*. (2015) discovered that TiO_2NPs promoted plant growth, promoted roots, and increased adhesive force between *B. amyloliquefaciens* and plants through the development of protective encapsulating interspecies. Also, Moaveni *et al*. (2011) claimed that TiO_2 nanoparticles may increase the amount of pigment and facilitate, by improving chlorophyll structure and light sorption, the transit of photosynthetic materials. By converting light energy into active electrons and chemical activity, nanoparticles extend the photosynthesis mechanism in the chloroplast. This process boosts the effectiveness of photosynthesis, activates the Rubisco activate complex, and promotes carbon photosynthesis in barley.

By interpreting these results through gene expression, an increase in gene expression was observed by applying these treatments. PR5 proteins interact with (1,3)-D-glucans of the fungal cell wall (Osmond *et al*., 2001). Inadequate fungal cell wall synthesis during hyphal extension is caused by the binding of PR5 proteins to nascent (1, 3)-D-glucans, which increases plasma membrane permeability (Bormann *et al*., 1999; Elsharkawy *et al*., 2022b). Therefore, we conclude from this study that these materials are efficient in combating the disease. There are also some previous studies that show that these materials have a role in increasing productivity (Raliya *et al*., 2015). Therefore, we recommend further study of these substances by clarifying the extent of their toxicity and their persistence in plants, thus generalizing their use in the field of plant diseases in the future.

CONCLUSION

Here, we used PCR-based approaches to quickly discover *Ustilago tritici* in health seeds that had not been found using standard methods such as the embryo count method. This study offers a viable approach for early detection and control of *U. tritici* in wheat harvests. This innovative PCR detection approach paired with TiO_2 and ZnO_2 nanoparticles offers various advantages for early management of loose smut in wheat. The immediate results of this assay will allow farmers to undertake control measures quickly, resulting in reduced disease transmission and crop losses. Furthermore, the introduction of nanoparticles increases the sensitivity of the detection approach, making it appropriate for routine screening of vast populations of wheat plants. Future research should focus on field validation and optimization of this method to ensure its practical application in disease management strategies.

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الكشف السريع بواسطة PCR لفطر *Ustilago tritici* والمكافحة المبكرة للتفحم السائب في القمح باستخدام الجسيمات النانوية TiO_2 وZnO_2

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مرض التفحم السائب، الذي يسببه فطر *Ustilago tritici*، هو مرض خطير يصيب القمح. لذا يتم فحص الأجنة والكشف المبكر بواسطة PCR عن هذا الفطر *Ustillago tritici* في البذور. وتم ايضا مكافحة مرض التفحم السائب باستخدام الجسيمات النانوية مثل TiO_2، ZnO_2. حيث لوحظ من بين 12 صنف قمح، ثلاثة أصناف هي سخا 61، سخا 93، جميزة 11 لديهم نسب كبيرة من الأجنة المصابة بـ *U. tritici*، تصل إلى 65%، مع شدة إصابة تصل إلى 15.3%. ولم يلاحظ إصابة في المعمل مع الاصناف سخا 95، سدس 14، ومصر1، ومصر 2، ومصر3؛ أما في الحقل فقد بلغت نسبة الإصابة إلى 30%. ولتعزيز الكشف المبكر عن البذور المصابة، تم تطوير تقنية حديثة باستخدام التكنولوجيا الحيوية PCR. وقد لوحظ باستخدام هذه الطريقة أن الأجنة في الأصناف التي كانت تعتبر في السابق غير مصابة بناء على الاختبارات المعملية هي في الواقع مصابة. وبالنسبة للمكافحة باستخدام الجسيمات النانوية TiO_2 وZnO_2 أدت إلى تقليل النمو الميسليومي لفطر *U. tritici*، مما أدى إلى تقليل الإصابة من 87% إلى أقل من 10% مقارنة بالكنترول، وهذا كان مشابهًا في الفاعلية مع مبيد الهاتريك. وبالدراسة ايضا اثبت زيادة التعبير الجيني لـ TaPR5 في النباتات المعاملة مقارنة بالنباتات غير المعاملة. ومن نتائج هذه الدراسة اعطت أدلة واضحة على الفعالية العالية لهذه الجسيمات النانوية في تعزيز نمو النبات ومكافحة المرض الفطري. لذا يمكن لهذه الجسيمات النانوية القوية أن تلعب دور هام كبدائل للمبيدات الفطرية في مكافحة مرض التفحم السائب في القمح.

الكلمات المفتاحية: قمح الخبز، *Ustilago tritici*، فحص الاجنه، التكنولوجيا الحيوية، المواد النانوية