

Article

Potential Applications of Chitosan-Coated Zinc Oxide Nanoparticles for Degrading Pesticide Residues in Environmental Soils

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Abstract: The precipitation process was applied to synthesize chitosan-coated zinc oxide nanoparticles (chitosan-ZnO NPs). Then, various characterization tools were used such as XDR, SEM, TEM, FTIR, and EDX. The use of these 50 nm chitosan-ZnO NPs in soil decontamination of thifluzamide and difenoconazole pesticide residues is being investigated. In two distinct soils, the effect of catalytic decontamination on pesticide residues was examined (sandy loam and sandy clay soils). The studies required two sets of pesticide concentrations. One set of samples was added to the chitosan-ZnO NPs catalyst, and the other set was studied without the addition of a catalyst. Photocatalytic studies were conducted under the sunlight in July. The soil samples were hand-spread in a glass dish to a height of 5 mm and sprayed with an aqueous solution of pesticide. From 8 a.m. to 5 p.m., these samples were exposed to sunlight in October 2021. We found that the best concentration of catalyst was 0.05%. The acquired samples were quantified using validated Ultra-Fast Liquid Chromatography (UFLC) with Photo Diode Array (PDA) detection. Kinetic parameters such as rate constant k and the degradation rate of pesticides DT50 have been calculated using Pesticide Residue Dissipation Data. The findings showed that the tested fungicides degenerate according to pseudo-first-order kinetics. Based on the findings, we concluded that photocatalytic degradation of pesticides in soils are faster than photolysis.



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Keywords: photocatalytic; chitosan-ZnO NPs; pesticide residues; rate constant; DT50

1. Introduction

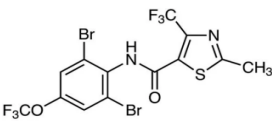
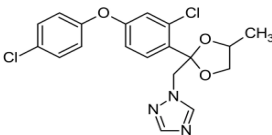
Nowadays, many pesticides are being used for crop cultivation. Mostly all the pesticides are toxic. Toxic pesticide residues have contaminated the ponds, rivers, and soil through field cultivation. Even the pesticide residues are found in the final harvest. When the crops are eaten by humans and animals, then pesticides are also consumed and ultimately there are many deaths. Therefore, in recent days, the environmental scientists are focusing their research on decontaminating pesticides in the environment as well as food products by using catalytic nanomaterials.

The environmental fate of agricultural pesticides is determined by the physical and chemical parameters of the soil system that are represented by organic matter, sand, silt, clay, moisture, and pH, as well as the vaporization, adsorption, and deterioration characteristics of pesticides, and accessibility to all water resources. Pesticide residues influence the soil's microbial population [1,2], are phytotoxic to succeeding crops, and enter water reservoirs [3] due to the compound's extended persistence or the development of bound residue in soil. Pesticide exposure has been associated to leukemia, lymphoma, and other types of cancer, as well as Parkinson's disease and developmental abnormalities in farmers, sprayers, and production employees. Pesticide residue buildup directly impacts not only the human species but also biodiversity [4] and animals [5] of an ecosystem.

Thifluzamide is a thiazole carboxamide fungicide developed by the Missouri Company of Monsanto, USA [6]. It is a non-volatile compound, but its leachability potential is high. It dissolves poorly in water (7.6 mg L^{-1} at 20°C) but the partition coefficient of octanol-water is high ($\log P = 4.16$ at $\text{pH } 7$, 20°C). Thifluzamide is a systematic fungicide and can be absorbed by roots and transferred to other plant parts. However, it is efficient in the management of a wide variety of basidiomycetes diseases as the foliar, soil, or seed treatment agent. In the tricarboxylic acid cycle of fungi, it can inhibit the succinic dehydrogenase enzyme [7]. Thifluzamide was found to have excellent anti-sheath blight activity in rice cultivars, and it has been approved for use in rice fields in many countries where rice is a primary staple food as in Asia and South America.

Difenoconazole is a broad-spectrum fungicide that controls a wide range of fungus, including those belonging to the Ascomycetes, Basidiomycetes, and Deuteromycetes families. It is used as a seed treatment, foliar spray, and systemic fungicide [8], and it is absorbed through the diseased plant's surface and administered to all parts of it. It has both a therapeutic and a protecting impact on the body. Winter wheat, rapeseed, Brussels sprouts, cabbage, broccoli/calabrese, and cauliflower can all be treated with difenoconazole. *Septoria tritici*, Brown Rust, Light Leaf Spot, Pod Spot, Ring Spot, and Stem Canker are among the fungi it controls [9]. The chemical structure of thifluzamide and difenoconazole along with their physicochemical properties are shown in Table 1.

Table 1. Physicochemical Properties of Thifluzamide and Difenoconazole.

Compound Name	Structure	Physicochemical Properties
Thifluzamide		Melting Point— 178°C Density (g mL^{-1})—2.0 Flashpoint ($^\circ\text{C}$)—177 Vapor pressure at 20°C (mPa)— 1.01×10^{-6}
Difenoconazole		Melting Point— 82.5°C Density (g mL^{-1})—1.37 Flashpoint ($^\circ\text{C}$)—285 Vapor pressure at 20°C (mPa)— 3.33×10^{-5}

Several decontamination techniques are used to preserve the environmental resources, and all types of planktons from pesticide's harmful residues. These techniques involve physical, chemical, and biological processes such as adsorption, biosorption, membrane filtration, aerosol, photochemical and electrochemical oxidation, and nano-bio redox reaction [1]. Heterogeneous photocatalysis is an advanced oxidation processes (AOPs) technology which is widely used for pesticide decontamination and other environment pollutions such as organic pollutants in soil and water [10,11]. Chitosan coating on metal oxide nanoparticles, particularly zinc oxide nanoparticles (ZnO NPs), has received much attention because of its less hazardous, eco-friendly, and varied uses [12,13]. ZnO NPs are well-known photocatalysts for pollutant degradation and mineralization [14–16]. Nanocomposites formed using chitosan and zinc oxide provide a new generation of biopolymer nanocomposites that are effective in controlling microbial infections and environmental pollutions. The size of ZnO nanoparticles (NPs) had an effect on the photocatalytic degradation of fungicides in a soil experiment. Several experiments concluded that the optimal size of the NPs for the maximum photocatalytic performance is determined by the compromise between the charge carrier recombination rate and specific surface area [17]. Ali H. Bashal et al. [18] described that chitosan is a biodegradable and biocompatible polysaccharide found in nature that can be used to immobilize metal oxide nanoparticles. In their work, ZnO NPs were doped within a chitosan matrix at different weight percentages and fabricated using a simple solution casting method.

In this regard, the present study was carried out to determine the conditions for the photocatalytic activity of chitosan-coated zinc oxide nanoparticles in soil decontamination of residues of thifluzamide and difenoconazole. Previously, many authors reported on metal-doped metal oxides being used as a catalyst for pesticide decontamination in environmental samples. We used biopolymer (chitosan)-coated ZnO NPs as photocatalysts in this study, which reduced catalyst toxicity. In our study, chitosan-coated ZnO NPs were used for fungicide decontamination as well as a good bio-fertilizer for soil decomposition.

2. Materials

2.1. Types of Equipment

Ultra-fast liquid chromatography (UFLC), Shimadzu UFLC HPLC system, Tokyo, Japan, with PDA detector that is interfaced with LabSolutions for analyzing pesticides residues with ZORBAX SB-C18, 3.5 μm , 150 mm $\times$ 4.6 mm, Agilent, (Santa Clara, CA, USA), Fourier transform infrared spectrometer FT/IR-6300 FTIR, JASCO, scanning electron microscope (SEM), JSM-IT510, JEOL USA Inc. (Peabody, MA, USA), transmission electron microscope (TEM), NEOARM, JEOL USA Inc., energy dispersive X-ray spectroscopy (EDX), EDX-3000, Ashlyn Chemunoor Industries Pvt. Ltd. (Thrissur, India), and X-ray diffractometer (XRD), XRDynamic 500, Rigaku (Tokyo, Japan), were used to characterize nanoparticles, Analytical balance, Mettler Toledo (Columbus, OH, USA).

2.2. Reagents and Chemicals

Chitosan (CAS number: 9012-76-4, deacetylation degree of 75%) and ZnO powder were obtained from Merck India Ltd., Mumbai. Thifluzamide and difenoconazole reference standard were procured from LGC Laboratories GmbH. Thifluzamide 24% SC and difenoconazole 24% EC were purchased from the local market. HPLC grade acetonitrile is provided by RANKEM, Chennai, India. Boric acid (BH_3O_3), sodium hydroxide (NaOH), orthophosphoric acid (H_3PO_4), ammonium chloride (NH_4Cl), magnesium chloride (MgCl_2), calcium chloride (CaCl_2), magnesium sulphate (MgSO_4), potassium dihydrogen orthophosphate (KH_2PO_4), disodium hydrogen orthophosphate (Na_2HPO_4), potassium chloride (KCl), GR grade ferric chloride were by RANKEM, Chennai, India.

3. Experimental

3.1. Preparation of Chitosan-ZnO NPs

Weight zinc cations were created by dissolving 1 g of ZnO powder in 100 mL of 1% acetic acid. This solution has 1 g of chitosan in it; 30 min were spent sonicating the mixture in ultra sonicator (Make: GT Sonic, Model: GT-F5 43 Khz, Duty cycle; 0.1 to 99.9%, Power supply; 220 to 240 V 50 Hz/110 V 60 Hz; amplitude 43,000 Hz); 1 M NaOH was added dropwise after magnetic stirring until the solution reached pH 10. For three hours, the mixture was heated in a water bath at 60 $^\circ\text{C}$. After filtering and numerous rounds of washing with distilled water, it was then dried for an hour at 50 $^\circ\text{C}$ in an oven [19–21].

3.2. Characterization

An XRD profile at room temperature was acquired from the synthesized chitosan-ZnO NPs. The sample was inspected to examine the phase purity and the crystal structure, and by using Scherrer equation, the crystallite size was determined from the XRD spectrum (XRDynamic 500, Rigaku). The SEM (JSM-IT510, JEOL USA Inc.) was used to investigate the surface morphology of chitosan-ZnO NPs. The size of particles has been calculated by TEM (NEOARM, JEOL USA Inc.). The FTIR spectrum was recorded to identify the functional groups. For detecting elements composing the prepared chitosan-ZnO NPs, energy-dispersive X-ray analysis (EDX-3000, Ashlyn Chemunoor Industries Pvt. Ltd., Kerala, India) was used.

3.3. Preparation of Standard Stock Solution

Five mg of pure thifluzamide and difenoconazole were weighted using an analytical balance then dropped into 5 mL volumetric flasks; the reference analytical standards stock solutions were prepared. Then, each flask's contents were dissolved with acetonitrile and filled up these flasks up to the mark.

3.4. Preparation of Test Item Solution

The formulation solution was prepared by adding the needed quantity of fungicides (Thifluzamide 24% SC and Difenoconazole 24% EC) to all 25 mL volumetric flasks, see Table 2. Then, the substances of each flask were dissolved in Milli pore water and filled up these flasks up to the mark.

Table 2. Fungicide composition and dosage of test item solution.

Fungicide Composition	Dosage
Thifluzamide 24% SC	T0-Control
Difenoconazole 24% EC	T1-1 mg/L

3.5. UFLC Separation Parameters

The Shimadzu UFLC HPLC system that had been used is described in Section 2.1. The column oven was kept at 40 °C and 20 µL of sample was injected. The two mobile phases were A (methanol) and B (0.1% orthophosphoric acid (90:10 (v/v))). The flow rate was maintained at 1.0 mL/min at a wavelength of 220 nm. The retention times of thifluzamide and difenoconazole were approximately 2.1 min and 2.7 min, respectively.

3.6. Preparation of Linearity Solutions

In order to check linearity, the fungicide stock solution was diluted in acetonitrile to create calibration solutions and by using six concentrations (5.0, 1.0, 0.5, 0.1, 0.03, and 0.01 mg/L) of thifluzamide and difenoconazole.

3.7. Accuracy and Limit of Quantification (LOQ)

Thifluzamide and difenoconazole were added in two different concentrations of 0.01 and 0.1 mg/L to four different soils in order to study recovery. At each concentration level, three replicate determinations were performed and two controls. The determination of the Limit of Quantification (LOQ) was conducted according to a recovery study.

3.8. Statistical Evaluation

The study considered the following parameters:

- For linearity study, regression equation ($Y = mX + C$, where m : slope and C : intercept), and correlation coefficient (R) were used.
- For precision study, mean, standard deviations (SD), relative standard deviation percentage (%RSD), and Horwitz limit = $2^{-(1-0.5 \times \log C)} \times 0.67$ were used.
- For recovery study, mean, standard deviations (SD), relative standard deviation percentage (%RSD), and Horwitz limit = $2^{-(1-0.5 \times \log C)} \times 0.68$ were used.
- For photocatalysis studied, the half-life (DT_{50}) was needed.

3.9. Experimental Soil Preparation

Sandy loam and sandy clay were considered as test soils. These soils were obtained from two different locations in Andhra Pradesh (Mugada and Vadada) to determine the dissipation of fungicide residues and evaluate the decontamination. A 2 mm sieve was used to filter the soil. The sieved soil was stored at −18 °C until the experiment started.

3.10. Procedures for Testing Soil Physicochemical Properties

3.10.1. Soil Texture

In a 1 L tall beaker, 20.0 g of air-dried soil was weighed and 50 mL of 30% *w/v* hydrogen peroxide (H_2O_2) was added and agitated. After 5 min of response time, the beaker was placed in a water bath at 80 °C for 30 min, covered with a watch glass. To prevent foaming, the mixing process was maintained. Another 20 mL of H_2O_2 was added to the beaker, which was then placed in the water bath at 50 °C for another 20 min. Then, the beaker's content was diluted to 150 mL with water and brought to the boil. The aim of the treatment with H_2O_2 was for enhancing the oxidization of the soil's organic matter.

3.10.2. Acid Treatment

The contents of the beaker were cooled, and 25 mL of 2 N HCl were added. After diluting to 250 mL, 10 mL of 0.1 M NH_4Cl , 30% NH_4OH , and 1 N NaOH were added, and the reaction was allowed to run for 1 h.

3.10.3. Clay and Silt Estimation

The beaker's content was poured into a 1000 mL cylinder without a spout and the volume was increased to 1000 mL then sealed with a rubber stopper and shaken. After removing the stopper and waiting 4 min, 20 mL of the content was pipetted and placed in a porcelain basin that was weighed. Following the evaporation of the liquid, the residue was dried at 105 °C, let to cool down and weighed. Finally, from the outcome, the percentage of clay and silt was recorded.

3.10.4. Clay Estimation

After 6 h of leaving the heterogeneous mixture alone, 20 mL was pipetted and evaporated. The residue was then dried at 105 °C, allowed to cool, and weighed. The percentage of clay was calculated based on the results.

3.10.5. Coarse and Fine Sand Estimation

After most of the supernatant liquid was emptied out of the cylinder, the deposit was transferred to a tall beaker filled with distilled water to a height of 10 cm from the bottom. After fully mixing, it was left aside for 4 min. After that, the hazy suspension was drained. The beaker was replaced with the appropriate amount of water, and the process was repeated until the liquid was clear. The residue was transferred to a pre-weighed porcelain basin, dried at 105 °C, then cooled and weighed. The percentage was calculated using total sand (coarse and fine).

3.10.6. Coarse Sand Estimation

To sieve with a 0.2 mm sieve, the coarse and fine sand fractions were removed from the porcelain basin. The sieve waste was put into a porcelain basin that had already been pre-weighed, dried at 105 °C, weighed, and the amount of coarse sand was determined. The percentage of fine sand was calculated by deducting the percentage of coarse sand from the total amount of sand.

3.10.7. pH

In a 100 mL beaker with 75 mL of distilled water, 30 g of soil was weighed. A glass rod was used to thoroughly mix the soil-water suspension. A pH meter was used to measure the pH.

3.10.8. Electrical Conductivity Determination

Thirty g of soil and 75 mL of distilled water were added to a 100 mL beaker after being weighed. After thoroughly mixing the soil and water, the suspension was given 0.5 h. While the electrode was immersed in the soil suspension, the electrical conductivity was measured.

3.10.9. Organic Carbon

In a 500 mL Erlenmeyer flask, 1.0 g of sieved soil (0.2 mm sieve) was added, followed by 10 mL of 1 N $\text{K}_2\text{Cr}_2\text{O}_7$, and then stirred. After 30 min, 20 mL of H_2SO_4 was progressively added. Another 200 mL of distilled water, 10 mL of H_3PO_4 , and 1 mL of diphenylamine indicator were added to prevent additional oxidation. The mixture was adjusted with 0.5 N $\text{FeH}_8\text{N}_2\text{O}_8\text{S}_2$ solutions until the blue color turned green. A sample without soil was also analyzed.

3.10.10. Cation Exchange Capacity Estimation

Ten g of air-dried soil was mixed with 50 mL of $\text{C}_2\text{H}_7\text{NO}_2$ solution in a 250 mL beaker, covered, and left overnight. The mixture was then passed through Whatman No. 3 filter paper that had been leached eight times with 30 mL of $\text{C}_2\text{H}_7\text{NO}_2$ solution. The filtrate was placed into a 250 mL volumetric flask, filled to the appropriate level, and retained to calculate the concentration of each exchangeable cation. A pinch of solid ammonium chloride was put on the filter paper, and it was leached with 250 mL of distilled water. The soil was thoroughly rinsed with alcohol until the filtrate was chloride-free after being entirely passed through the filter paper with 60% alcohol in the beaker. The soil filter paper was carefully removed and placed in a distillation flask. The flask was halfway full when it was filled with distilled water (about 400 mL); 25 mL of 0.1 N H_2SO_4 and two drops of methyl red indicator were added to a 500 mL ice tumbler. The tumbler was then put beneath the feed hose, ensuring that the hose made contact with the 0.1 N H_2SO_4 surface; 10 mL of 40% NaOH was added to the flask holding the dirt. The flask was immediately capped, and distillation began, with ammonia levels tested. The feed hose was washed with distilled water in the same tumbler until the distillate was ammonia-free. After then, the tumbler was withdrawn and titrated against 0.1 N KOH. The endpoint was the appearance of straw yellow from red.

3.11. Photocatalytic Studies

The research was conducted in normal weather conditions under sunlight in the month of July. To acquire a homogeneous 0.3% (*w/w*) of chitosan-ZnO NPs in soil, soil samples were thoroughly mixed with an aqueous suspension of chitosan-ZnO NPs. In a glass dish, the loaded chitosan-ZnO NPs soil was distributed to a height of 5 mm. Aqueous fungicide mixture diluted in 50 mL of distilled water was sprayed. A 1 L high density polyethylene bottle sprayer was used. To evaporate the water molecules from the soil, it was heated to 110 °C in the oven for two hours. Samples from each soil type were placed in the sun, and 3 samples were kept. To identify the presence of fungicides in treated soil and the absence of fungicides in untreated soil, chitosan-ZnO NPs loaded soil sprayed with distilled water was retained as an untreated control. The soil samples were exposed to sunlight at temperatures ranging from 28 to 40 °C during the day. A LUX meter was used to calculate the intensity of solar radiation during the exposure time.

Sampling of Soil

Soil samples were taken from the glass dish at different locations on different occasions after exposure to sunlight (0, 8, 20, 30, 48, 72, and 90 h) for photocatalytic experiments. The soil samples were thoroughly mixed, and the fungicide was extracted from a 50 g subsample.

4. Results and Discussion

4.1. XRD Pattern

Figure 1a shows the X-ray diffraction patterns of ZnO nanoparticles (ZnO NPs) and chitosan-ZnO nanocomposites, respectively. All diffraction peaks of ZnO NPs were well indexed in good agreement with the hexagonal (space group = $\text{P6}_3/\text{mmc}$) structure of JCPDS Card no. 36-1451. The broad diffraction peak at $2\theta \sim 20^\circ$ appeared in the diffraction pattern of chitosan-ZnO nanocomposites assigned to semi-crystalline chitosan [22] and confirmed that the hexagonal ZnO NPs were well blended into the chitosan matrix. The

peak positions and fullwidth at half maxima (FWHM) of the chitosan-ZnO nanocomposites appeared to be slightly altered which indicate the modification in the ZnO particles size during composite formation. The crystallite size of the ZnO NPs and chitosan-ZnO nanocomposites calculated using the Scherrer formula was found to be 43 nm and 18 nm, respectively. The structural refinement was performed with the FullProf software in order to foresee the effect of chitosan-ZnO interactions on the unit cell parameters.

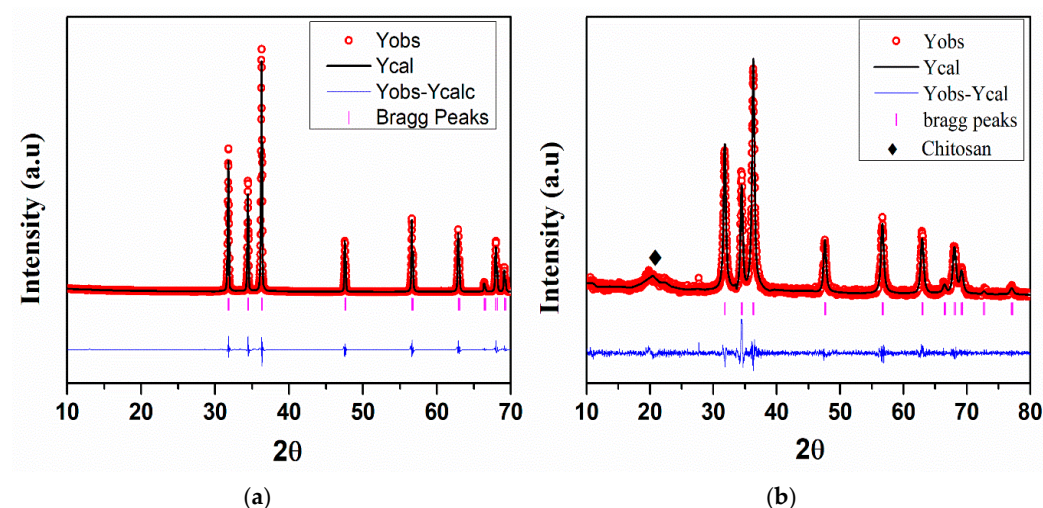


Figure 1. XRD pattern of ZnO NPs (a) and Chitosan-ZnO NPs (b).

Table 3 shows the refined unit cell parameters of ZnO NPs and chitosan-ZnO nanocomposites. The unit cell parameters of the hexagonal cell remained unchanged after the incorporation of ZnO into the chitosan matrix. However, slight elongation of O-Zn-O Bond length and Bond angle along the c-axis was noticed from the structural refinement of the chitosan-ZnO nanocomposites.

Based on the diffraction patterns of both the samples, it can be established here that the nanosized ZnO particles homogeneously distributed throughout the chitosan matrix and the addition of ZnO into polymer chains significantly affect the lattice structure and crystallinity of the wurtzite unit cell. However, the crystallite size of the ZnO particles decreased with the formation of composites. Further, it has also been well known that for ZnO as a filler has more potential compared to other nanoparticles to create strong bonding interaction with the polymer matrix. Therefore, the very marginal elongation observed for O-Zn-O lattice site and reduction in the crystallite size for chitosan-ZnO composites may be attributed to a strong interfacial stretching force between polymer chains and ZnO surface that contributed to slight distortion of the surface oxygen and perhaps prevented the ZnO particles to aggregate in the form of large crystallites during the composite formation.

4.2. FTIR and EDX Analysis of Chitosan-ZnO NPs

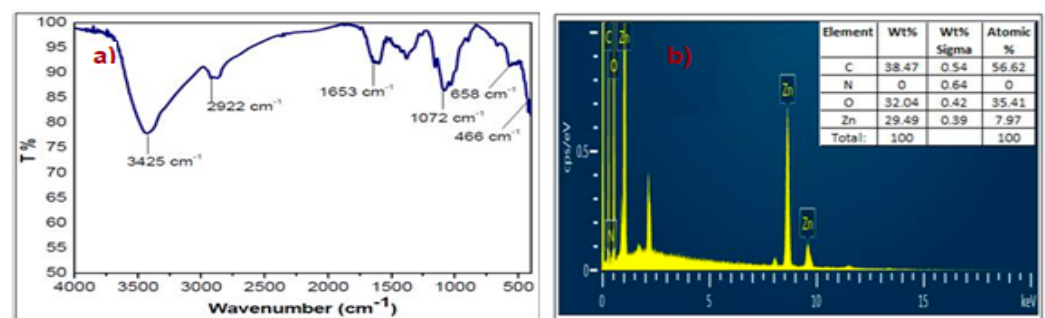
The FTIR spectrum of chitosan-ZnO nanocomposite is shown in Figure 2a. For pure chitosan, the characteristic bands at 3425 cm^{-1} and 2922 cm^{-1} were attributed to the stretching vibration of -OH groups and C-H groups, respectively. The stretching vibrations of the C=O and C-N groups were allocated to the bands at 1653 cm^{-1} and 1072 cm^{-1} , respectively. The vibration of O-Zn-O groups was attributed to the band in the FTIR spectra of chitosan-ZnO nanocomposite [22] between 580 and 400 cm^{-1} .

The elemental composition of chitosan-ZnO NPs was probed by EDX as shown in Figure 2b. The chitosan-ZnO NPs exhibit four elemental peaks, one for O at 0.8 keV and three for Zn at 1.0 keV, 8.5 keV, and 9.7 keV. Carbon element C located at 0.08 keV. From EDX data, the weight ratio of C:O:Zn was about 38.47:32.04:29.49, respectively. The sample consists only of the elements C, O, and Zn.

Table 3. Unit cell parameters of ZnO NPs and Chitosan-ZnO nanocomposites.

Chitosan-ZnO Nanocomposites							
Atom	x	y	z	Occ.	B _{iso}	Site	Sym.
Zn	0.33	0.67	0.73	1	0.042	2b	3m.
O	0.33	0.67	1.12	1.15	0.055	2b	3m.
Unit cell parameters	a = b = 3.25 Å c = 5.203 Å α = β = 90° γ = 120°						
Unit cell volume	47.54 Å ³						
R _p (%)	18.3						
R _{wp} (%)	24.7						
χ ²	12.3						
Zn-O (8)	2.02 Å						
O-Zn-O(10)	107.11°						
Pristine ZnO NPs							
Atom	x	y	z	Occ.	B _{iso}	Site	Sym.
Zn	0.33	0.67	0.002	1	0.01	2b	3m.
O	0.33	0.67	0.38	1.09	0.027	2b	3m.
Unit cell parameters	a = b = 3.25 Å c = 5.206 Å α = β = 90° γ = 120°						
Unit cell volume	47.6 Å ³						
R _p (%)	16.1						
R _{wp} (%)	19.4						
χ ²	4.02						
Zn-O (8)	1.99 Å						
O-Zn-O(10)	108.09°						

x, y, z: Atomic parameters, a, b, c, α , β , γ : Unit cell parameters, R_p: Profile residual, R_{wp}: Weighted profile residual, χ^2 : Goodness of fit, Occ.: Occupancy, B_{iso}: Isotropic thermal factor, Site: Wyckoff site.

**Figure 2.** (a) FTIR spectra; (b) EDX pattern of Chitosan-ZnO NPs.

4.3. SEM and TEM Analysis of Chitosan-ZnO NPs

The surface characteristics of chitosan-ZnO NPs were examined by SEM and the image obtained is shown in Figure 3a. The SEM image shows that the chitosan-ZnO NPs have smooth surfaces [23,24]. However, it shows that some of the NPs were agglomerated. Figure 3b displays the TEM image of chitosan-ZnO NPs. It reveals that chitosan-ZnO NPs are uniform and spherical. The calculated average particle size was found to be 50 nm with Image-J software.

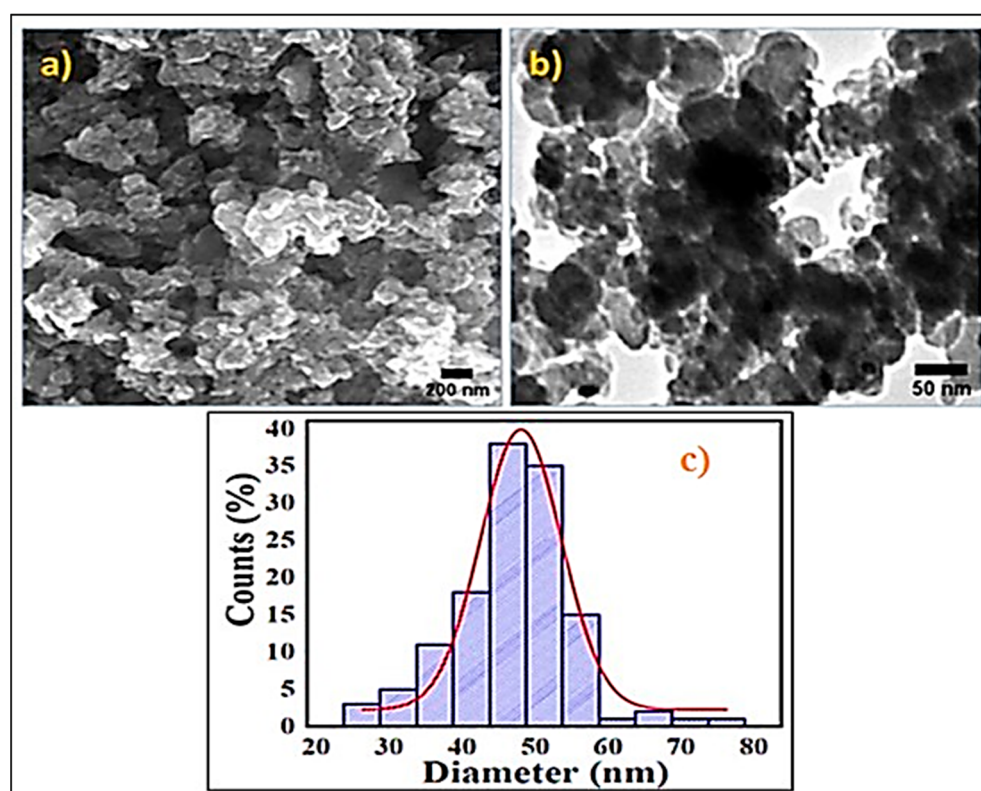


Figure 3. (a) SEM image of Chitosan-ZnO NPs; (b) TEM Image of Chitosan-ZnO NPs; (c) Size distribution curve.

4.4. Physicochemical Characteristics of Soil

The calculation of soil physicochemical properties is presented in Table 4.

Table 4. Calculation of soil physicochemical properties.

Soil Type	Sandy Loam	Sandy Clay
Parameter	Results	
Color	Very dark grayish brown	Very dark grayish brown
pH	7.2	6.8
Electrical conductivity C _{μmhos/cm} (10% suspension))	107.3	118.1
Organic carbon content (%)	0.5	4.5
Cation exchange capacity (meq/100 gm of soil)	30.1	20.9
Texture		
Sand percentage	56	13
Silt percentage	19	59
Clay percentage	25	28

4.5. Linearity

The implemented processes were found to be linear with an appropriate correlation coefficient when tested in the range 5.0–0.01 mg/L for thifluzamide and difenoconazole. Based on a 10:1 peak to noise ratio, the Limit of Quantification (LOQ) was determined to be 0.03 mg/L. Table 5 summarizes the correlation coefficient (R) and linear regression equation ($Y = mX + C$) that were created by graphing various concentrations of calibration solutions

vs. the observed one. Figure 4 depicts calibration curves. Both pesticides demonstrated good linearity, with R^2 values of 0.9999 and 1 for difenoconazole and thifluzamide, respectively.

Table 5. Calibration details—thifluzamide and difenoconazole.

Concentration (mg/L)	Peak Area of Thifluzamide (mAU)	Peak Area of Difenoconazole (mAU)
5	152,874	185,474
1	30,587	37,895
0.5	15,260	20,025
0.1	3157	3805
0.03	1123	1236
0.01	334	412

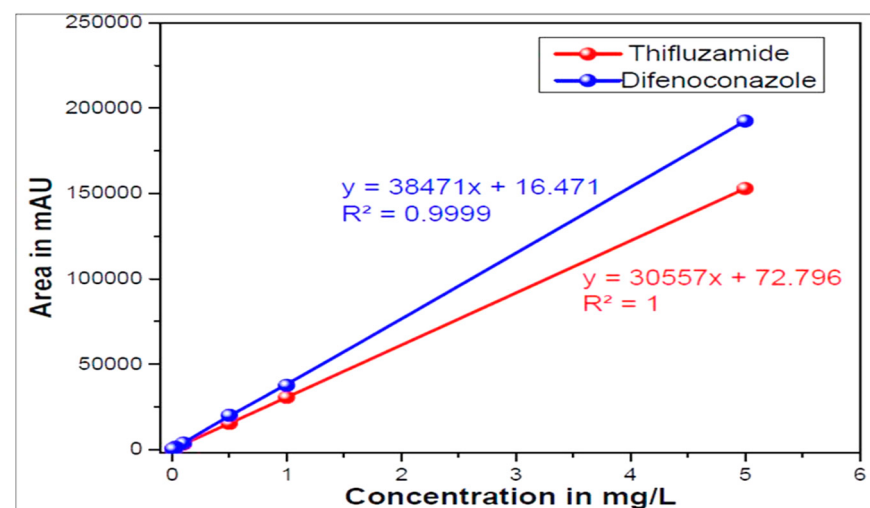


Figure 4. Calibration curve of linearity standards.

4.6. Accuracy and Repeability

When $10 \times \text{LOQ}$ recovery sample was injected five times into the HPLC, precision with an allowed threshold of 10% RSD was reached. Figure 5 shows the mean, standard deviation (SD), and relative standard deviation percentage (% RSD). In two separate soils, an acceptable recovery range for fungicides (80–110%) was confirmed. The LOQ was set at 0.01 mg/L based on a peak-to-noise ratio of 10:1 [1,25,26]. All necessary formulas and parameters are listed below [27]:

$$\text{Residue content } (\mu\text{g/g}) = \frac{P1 \times V_s \times C}{P2 \times W} \times F \quad (1)$$

where P1 is active content's peak area in the sample ($\mu\text{V} \times \text{s}$); V_s is the sample's volume (mL); C is the standard solution concentration (mg/L); P2 is the active content's peak area in the reference solution ($\mu\text{V} \times \text{s}$); W is the sample's weight (g), and F is the dilution factor.

$$\text{Recovery \%} = \frac{\text{Recovered residue} \times 100}{\text{Fortified concentration}} \quad (2)$$

$$\% \text{RSD} = \frac{\text{SD} \times 100}{\text{Mean}} \quad (3)$$

$$\text{Horwitz Limit} = 2^{-(1-0.5 \times \log C)} \times 0.67, \quad (4)$$

where C is the concentration of analyte.

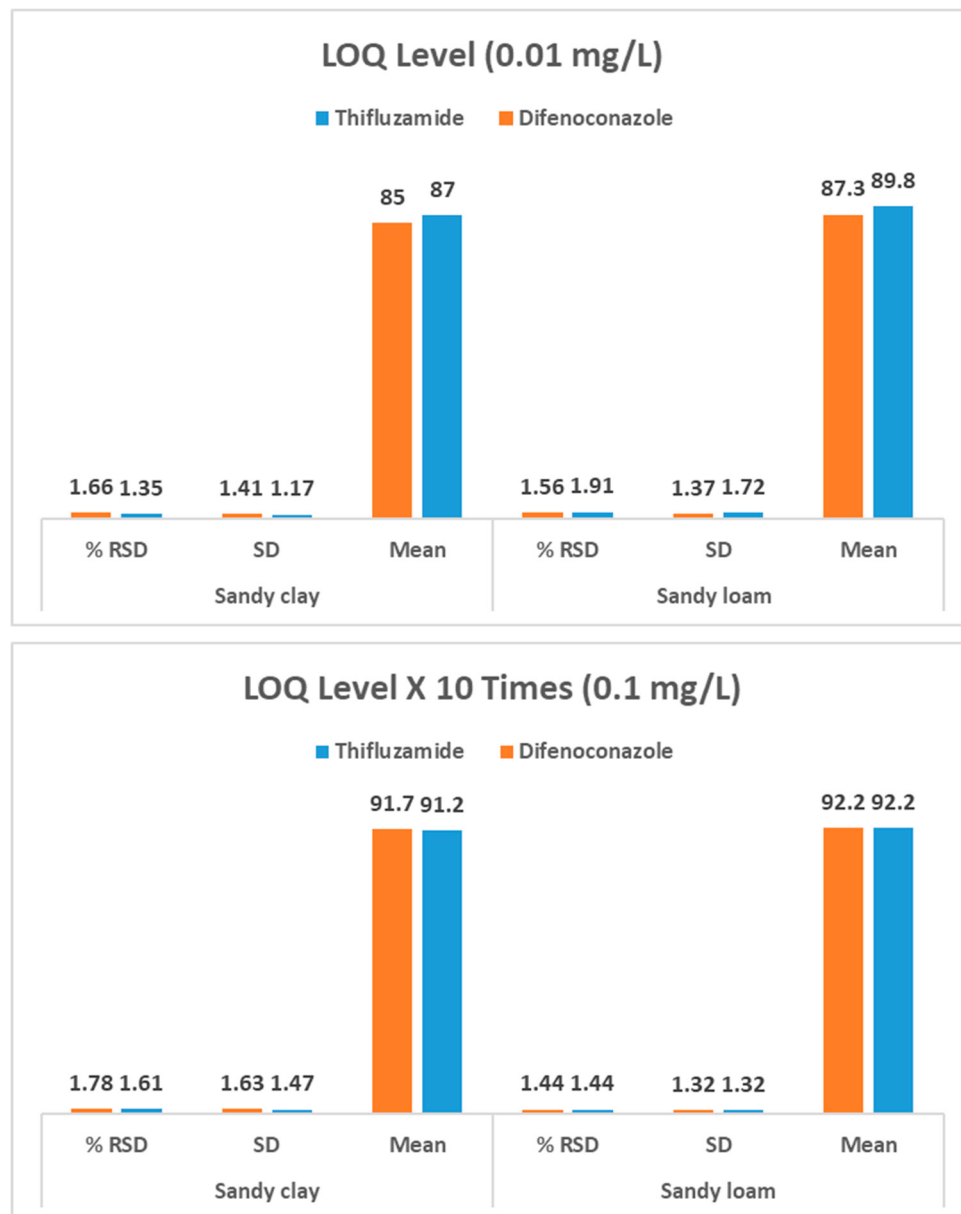


Figure 5. Recovery details of fungicides in soil.

4.7. Photocatalytic Decontamination of Pesticide in Soil

In order to ensure the establishment of adsorption/desorption equilibrium of the fungicides on the surface of the sample, the samples with fungicides were kept in the dark for 1 h as shown in Figure 6. It was observed that the adsorption of fungicides on the surface of the samples is limited, thus, the equilibrium has been reached. Furthermore, a negligible degradation of the fungicides treated with photocatalyst in the dark was observed. The amount of degradation was found to be close to the value of the blank sample which comprising fungicides illuminated with the light without a photocatalyst. To study the effect of photocatalyst on fungicides, samples were treated in the light and the results are presented in Figure 6a,b. ZnO without chitosan was also used to compare the results and to analyze the role of chitosan in the photocatalysis. Figure 6a shows the photocatalytic degradation of Thifluzamide using ZnO, while Figure 6b depicts the degradation of difenoconazole with ZnO, respectively.

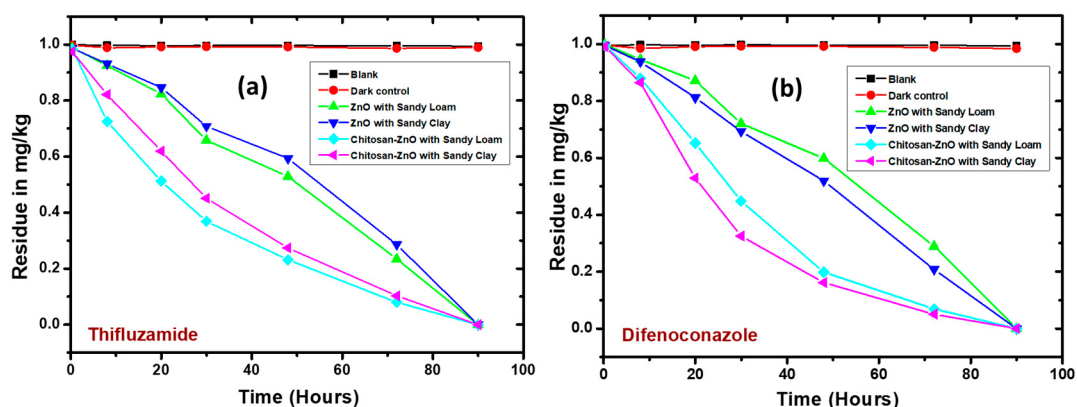


Figure 6. The dissipation curves of fungicide photocatalytic remediation of soils in sunlight by (a) thifluzamide and (b) difenoconazole.

Thifluzamide residue was 0.985 mg/kg for sandy loam and 0.975 mg/kg for sandy clay soil with 0.05% load of chitosan-ZnO catalyst in 0.3 h analysis of thifluzamide fortified soil, respectively. After 8 h in sandy loam and sandy clay soil with a 0.05% chitosan-ZnO catalyst load, thifluzamide residues were dissipated to 0.725 mg/kg and 0.821 mg/kg, respectively. After 20 h with a 0.05% load of chitosan-ZnO catalyst, the thifluzamide residues were dissipated to 0.512 mg/kg and 0.619 mg/kg, respectively. The thifluzamide residues were dissipated to 0.369 mg/kg and 0.451 mg/kg after 30 h with a 0.05% chitosan-ZnO catalyst load, respectively. The dissipation of thifluzamide residues to 0.231 mg/kg and 0.274 mg/kg took place after 48 h with a 0.05% chitosan-ZnO catalyst load, respectively. The thifluzamide residues were dissipated to 0.08 mg/kg and 0.103 mg/kg with a 0.05% chitosan-ZnO catalyst's load after 72 h and were completely degraded (BDL—below detectable level) after 90 h. For ZnO, the degradation is much less than that of chitosan-ZnO which could be clearly seen from Figure 6a.

The difenoconazole residue in 0.3 h analysis of difenoconazole fortified soil, on the other hand, was 0.994 mg/kg for sandy loam and 0.991 mg/kg for sandy clay soil with 0.05% load of chitosan-ZnO catalyst, respectively. Difenoconazole residues were dissipated to 0.880 mg/kg and 0.864 mg/kg after 8 h in sandy loam and sandy clay soil with a 0.05% chitosan-ZnO catalyst load, respectively. With a 0.05% dosage of catalyst, the difenoconazole residues were broken down after 20 h to 0.652 mg/kg and 0.529 mg/kg, respectively. With a 0.05% load of chitosan-ZnO catalyst, the difenoconazole residues were broken down after 30 h to 0.448 mg/kg and 0.325 mg/kg, respectively. With a 0.05% chitosan-ZnO catalyst load, the difenoconazole residues were broken down after 48 h to 0.198 mg/kg and 0.161 mg/kg, respectively. The BDL was reached at 90 h after the difenoconazole residues had been broken down to 0.0069 mg/kg and 0.050 mg/kg, respectively, with a 0.05% chitosan-ZnO catalyst load. It was found that chitosan-ZnO provided higher degradation of difenoconazole residue than that of pure ZnO. The results for photocatalytic studies are summarized in Table 6 and they are shown in Figure 6a,b, while the chromatogram's results are shown in Figure 7a–d. During the exposure time, the intensity of solar radiation was measured. The information is shown in Table 7.

Table 6. Data on dissipation for photocatalytic fungicide remediation in soil under sunlight.

Occasion (Hours)	Residues (mg/kg)	
	Sandy Loam	Sandy Clay
Thifluzamide		
0.3	0.985	0.975
8	0.725	0.821
20	0.512	0.619

Table 6. Cont.

Occasion (Hours)	Residues (mg/kg)	
	Sandy Loam	Sandy Clay
Thiﬂuzamide		
30	0.369	0.451
48	0.231	0.274
72	0.08	0.103
90	BDL	BDL
Difenoconazole		
0.3	0.994	0.991
8	0.880	0.864
20	0.652	0.529
30	0.448	0.325
48	0.198	0.161
72	0.069	0.050
90	BDL	BDL

BDL—Below the detectable level.

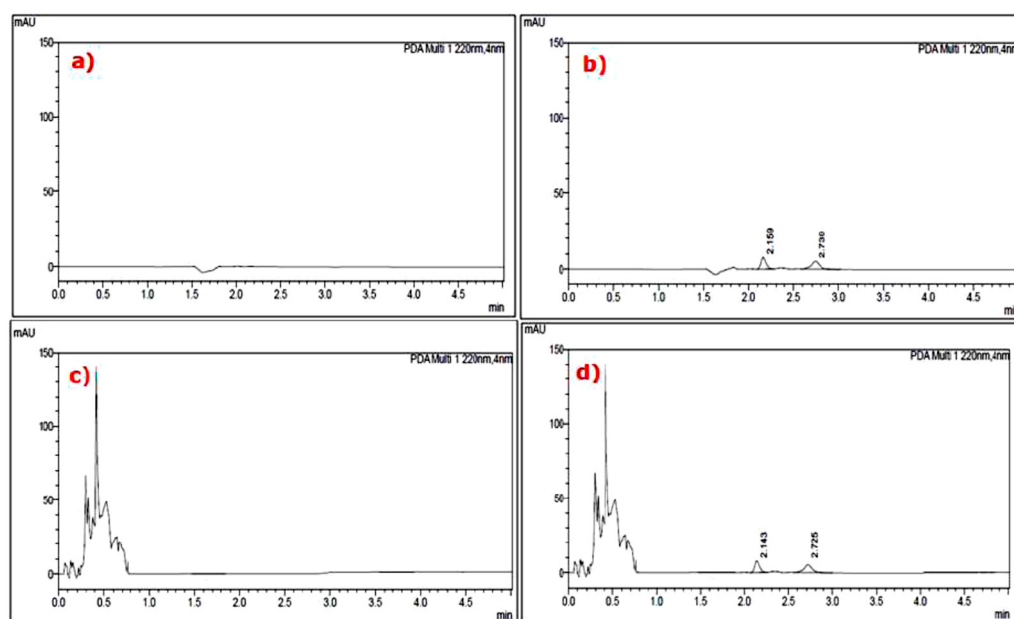


Figure 7. The typical chromatograms of the 30th hour show the following: (a) Sandy Loam control; (b) Sandy Loam pesticide residues; (c) Sandy Clay control; (d) Sandy Clay pesticide residue.

Table 7. The intensity of solar radiation measure at different time intervals (hours).

Time (Hours)	Solar Intensity (LUX)
0.3 (12 October 2021; 10:00 a.m.)	85,787
8 (12 October 2021; 6:00 p.m.)	9525
20 (13 October 2021; 6:00 a.m.)	11,748
30 (13 October 2021; 4:00 p.m.)	54,124
48 (14 October 2021; 10:00 a.m.)	87,124
72 (12 October 2021; 10:00 a.m.)	84,718
90 (12 October 2021; 10:00 a.m.)	89,528

The results demonstrate that the decontamination of fungicides in chitosan-ZnO-coated soil followed pseudo-first-order kinetics when residue levels were estimated over time using the first-order kinetic equation. The pseudo-first-order kinetics take the form of [28]:

$$-\ln\left(\frac{C_t}{C_0}\right) = k t \quad (5)$$

$$t_{1/2} = DT_{50} = \frac{\ln 2}{k} \quad (6)$$

$$k = \frac{2.303}{t} \times \log_{10}\left(\frac{C_0}{C_t}\right) \quad (7)$$

where C_0 is fungicide concentration at times zero; C_t is fungicide concentration at times t ; k is the rate constant, and DT_{50} is half-life.

The kinetic parameters for photocatalytic decontamination, such as the rate constant (k) and decontamination threshold (DT_{50}), are provided in Tables 8 and 9.

Table 8. Kinetic parameters for thifluzamide photocatalytic decontamination in soil under sunlight.

Occasion (Hours)	Sandy Loam				Sandy Clay			
	Residue in mg/L	Log Values	Results		Residue in mg/L	Log Values	Results	
0.3	0.985	−0.0066	Slope	−0.015	0.975	−0.0110	Slope	−0.013
8	0.725	−0.1397	Half-life (DT50) (Hr)	20.54	0.821	−0.0857	Half-life (DT50) (Hr)	22.33
20	0.512	−0.2907	C (s ^{−1})	0.002	0.619	−0.2083	C (s ^{−1})	0.034
30	0.369	−0.4330	R	0.996	0.451	−0.3458	R	0.994
48	0.231	−0.6364			0.161	−0.5622		
72	0.08	−1.0969			0.050	−0.9872		
90	BDL	BDL			BDL	BDL		

Table 9. Kinetic parameters for difenoconazole photocatalytic remediation in soil under sunlight.

Occasion (Hours)	Sandy Loam				Sandy Clay			
	Residue in mg/L	Log Values	Results		Residue in mg/L	Log Values	Results	
0.3	0.994	−0.0026	Slope	−0.017	0.991	−0.0039	Slope	−0.018
8	0.880	−0.0553	Half-life (DT50) (Hr)	18.14	0.864	−0.0635	Half-life (DT50) (Hr)	16.34
20	0.652	−0.1858	C (s ^{−1})	0.084	0.529	−0.2765	C (s ^{−1})	0.060
30	0.448	−0.3487	R	0.991	0.325	−0.4881	R	0.997
48	0.198	−0.7033			0.161	−0.7932		
72	0.069	−1.1612			0.050	−1.3010		
90	BDL	BDL			BDL	BDL		

Previously, many authors reported metal-doped metal oxides used as catalysts for decontamination of pesticides in environmental samples. In this study, we used biopolymer (chitosan)-coated ZnO NPs as photocatalysts which reduced catalyst toxicity. In our research, chitosan-coated ZnO NPs were used for decontamination of fungicides as well as acting as a good bio-fertilizer for soil to decompose easily.

5. Conclusions

The chitosan-ZnO NPs were discovered to be a good catalyst for decontaminating the residues of two types of fungicides, thifluzamide and difenoconazole, in several soil samples. Without a catalyst, the compounds lasted for several days. The chromatographic analysis of three distinct types of buffers needed a very brief run time, and the mobile phase of acetonitrile and 0.1% orthophosphoric acid demonstrated good separation and resolution. It was evident from the performed photocatalytic studies of pesticides at various conditions

that with chitosan-ZnO NPs, the activity is dramatically increased when the time frame is fixed for a predetermined number of hours, whereas activity was not observed without chitosan-ZnO NPs, even though experiments were conducted over a number of days.

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