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STUDY ON PREPARATION AND PREVENTION EFFICIENCY FOR *Colletotrichum truncatum* FUNGES CAUSING ANTHRACNOSE IN SOYBEAN PLANTS (*Glycine max*) OF OLIGOCHITOSAN-Zn^{2+} COMPLEXES

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INTRODUCTION

1. The urgency of the thesis

Oligochitosan (OC) is a natural polysaccharide derivative having a low molecular weight (Mw) prepared by degrading the chitosan (CTS) with prevalent agents such as enzymes, chemicals, and ionizing radiation. OC has been researched and proven to be non-toxic, capable of inhibiting pathogenic microorganisms and eliciting disease-resistance and preventive effects on many plant diseases. OCs with Mw < 10 kDa have exhibited effective disease resistance-stimulating activity and are recognized as being better than CTS.

OC inheres to an ability to form complexes with many metal ions such as Cu^{2+}, Zn^{2+}, Mn^{2+}, Fe^{2+}, and others. In complex form, the antimicrobial activities are increased significantly. Among of them, the OC-Zn^{2+} complex has attracted much research and application to prevent plant diseases. Zinc is an essential trace element for growing and developing and one of the key agents that plays a pivotal role in enhancing plant defense responses. The complex showed an antimicrobial ability 2 – 8 times higher than OC and 4 – 16 times for Zn^{2+}. In addition, applying zinc in plants also increases the accumulation in agricultural products of essential micronutrients. Therefore, OC-Zn^{2+} complex is considered a potential for application in agriculture.

In Vietnam, the soybean plant (*Glycine max*) is a prominent crop. According to statistics in 2020, the soybean growing area is 166,000 ha with an output of 265,000 tons but only enough to supply 8 – 10% of domestic demand. Global trends in safe agricultural production in general and soybeans in particular, the control of frequent diseases caused by *Colletotrichum truncatum* (*C. truncatum*), *Fusarium solani*, etc. with agrochemicals such as OC-Zn^{2+} complex is necessary. The impact of the complex on the type of each crop or pathogenic microorganism is certainly dissimilar. However, up to now, the preventability and eliciting disease-resistant chitinase for anthracnose on soybeans caused by the *C. truncatum*

of the OC-Zn^{2+} complex has not been investigated in shedding some light.

For the above mentions, we chose and carried out the project "Study on preparation and prevention efficiency for *Colletotrichum truncatum* fungus causing anthracnose in soybean plants (*Glycine max*) of oligochitosan-Zn^{2+} complexes".

2. The objectives of the thesis

The pursuing object of the thesis is to research the production of OC with a Mw less than 10 kDa using Co-60 gamma-ray irradiation combined with low-concentration H_2O_2, used to manufacture OC-Zn^{2+} complexes. And investigate the biological effects of OC-Zn^{2+} complex on preventing anthracnose caused by *C. truncatum* fungus, eliciting disease-resistant chitinase enzyme and stimulating growth on soybean plants.

3. The main research contents in the thesis

Research on preparing OC with an Mw from 2 – 8 kDa using the Co-60 gamma-ray irradiation combined with H_2O_2 and determine characteristics.

Research on synthetizing of OC-Zn^{2+} complex and define of properties.

Appraise on the inhibitory effect of OC-Zn^{2+} complex for *C. truncatum* fungus under laboratory conditions (*in vitro*).

Research on the effect of chitinase induction and growth parameters of soybean plants treated with OC-Zn^{2+} complex and artificially infected with the fungus *C. truncatum* causing anthracnose in a greenhouse experiment.

Evaluate the disease preventability and agronomic effectiveness of the OC-Zn^{2+} on soybean plants naturally cultivated in the experiment field.

Chapter 1. LITERATURE REVIEW

1.1. Chitosan, oligochitosan and their ability to control plant diseases

1.1.1. Chitosan

Chitosan (CTS), a deacetylated derivative of chitin, is mainly found in the shells of crustaceans, insects, etc. They have been widely applied in many

fields due to their unique properties, such as non-toxic, degradable, biocompatible, and antimicrobial. Structurally, the CTS is a copolymer consisting of D-glucosamine (GluN) and N–acetyl-D-glucosamine (GluNAc) units, of which the amount of GluN accounts for at least 50%.

The antimicrobial mechanism of CTS has been assumed to relate to disrupting bacterial cell membranes by electrostatic interactions, concurrently affected DNA leading to dysfunctional replication of DNA, or binding to necessary nutrients indispensable for microbial growth, which all these activities lead to the death of microorganisms. CTS is also considered a plant vaccine. It has supported plants to increase resistance capability and stimulate biosynthesis of phytoalexin or disease-disintegrating enzymes such as chitinase and glucanase against invading harmful microorganisms.

Recently, there have been some studies using CTS to control plant diseases. The CTS with 1 mg/ml in concentration was effectively antifungal of *Phytophthora infestans* damaged tomatoes and *Phytophthora palmivora* caused rubber defoliation disease. Appling CTS of 3 mg/ml inhibited *Fusarium solani* f. sp. *glycines* and antifungal *F. sulphureum* that damages potato roots. CTS and their derivatives have a resistance ability against microorganisms in broad-spectrum, so they have the potential to be used to prevent bacteria, fungi, and nematodes that damage seriously on plants.

1.1.2. Oligochitosan

Oligochitosan (OC) is a degraded CTS product with Mw < 10 kDa, prepared by scissoring β-1,4-glycoside bonds in the CTS molecules by agents such as chemicals, enzymes, and ionizing radiation (Co-60 gamma rays, electron beam). Compared to CTS, OC dissolves better due to its short molecular chain and many amino groups in the free.

The antimicrobial activity of OC has been assumed to be higher than that of CTS and depends on the Mw and type of microorganisms. OC's ability to stimulate plants to produce phytoalexins and disease-eliminating enzyme

proteins in many crops has been studied already. Burkhanova (2007) reported that OC with Mw 5 – 10 kDa exhibited a high controllability for root rot disease in wheat. Ozeretskovskaya *et al.* (2006) demonstrated that OC with Mw from 2 – 6 kDa effectively prevented late blight disease in potatoes better than CTS with high Mw. Li *et al.* (2016) revealed that OC with 1 mg/ml in concentration can restrain plant stem rot caused by *Fusarium sambucinum* fungus. Du *et al.* (2015) disclosed that using OC Mw ~5 kDa at 150 ppm and then spraying the fungus *Neoscytalidium dimidiatum* on dragon fruit plants stimulated chitinase enzyme reaching a level of 34.9 UI/g was higher than the water spray control (19.1 UI/g) while reduced disease rates and disease index. Tuan *et al.* (2019) also found that OC with Mw 3 kDa supported dragon fruit for inducing the enzyme chitinase and achieving a higher effectiveness in preventing brown spot disease than OC with Mw of 5 kDa or 7 kDa. The ability to stimulate plants to produce antibiotics (phytoalexin) and antibody enzyme proteins (chitinase,..) against pathogenic microorganisms depended on Mw of OC. When their Mw was less than 5 kDa, OC showed an evidential disease resistance-stimulating effect.

1.2. OC-Zn^{2+} complex and their ability to control plant diseases

CTS and OC exhibit strong ability to form complexes with many metal ions, such as Cu^{2+}, Zn^{2+}, Fe^{2+}, etc., by coordinating bonds with amino ($–NH_2$) and hydroxyl (–OH) groups. OC-Zn^{2+} complex is interested in research and application to plants. In plants, zinc participated in the process of growth and development, as well as stimulating immunity. Zinc affects the functioning of micro-organisms and inactivates them, and stimulates plants to activate defense reactions such as increasing the induction of salicylic acid, jasmonic acid, or ethylene, and increases lignin biosynthesis to reinforce cell walls, activating zinc-containing enzymes or proteins to against pathogen attacks.

Owing to the OC-Zn^{2+} complex being made up of two components that inhered the ability to anti-microorganisms and stimulate disease resistance,

this complex is highly effective when used to control plant diseases. Wang *et al.* (2004) demonstrated that the CTS-Zn^{2+} complex has antibacterial and antifungal effects 2 – 8 times higher than CTS and 4 – 16 times for Zn^{2+}. When corn plants were applied a CTS-Zn^{2+} complex, were increased disease resistance response and increased corn yield by 20 – 40%. Ibrahim *et al.* (2015) demonstrated that the CTS-Zn^{2+} complex enhanced growth, nutrient absorption efficiency, stress tolerance, and increased productivity of bean plants from 25% – 33%. Hien *et al.* (2008) announced that the use of a mixture of (OC + Zn^{2+}) reduced the disease severity of the leaf by 36%, stem by 66%, and increased sugarcane yield by 12%. However, up to now, the disease prevention effect through the resistance-stimulating efficiency of the OC-Zn^{2+} complex on soybean diseases has almost not been published.

1.3. OC preparation methods

OC was prepared mainly by degrading methods: chemical (H_2O_2, strong acid), physical (microwave radiation, ultraviolet rays, gamma-ray radiation, electron beam, etc.), biological (using enzymes cellulase, chitinase, etc.), or a combination of them. In this dissertation, the Co-60 gamma-irradiating method combined with H_2O_2 used to make OC has the following mechanism: first, the radiolysis reaction of H_2O and H_2O_2 forms active free radicals.

$$H_2O \xrightarrow{\text{tia } \gamma} e^-_{aq}, H^\bullet, {}^\bullet OH, H_2O_2, H_2, H_3O^+, .. \quad (1.1)$$

$$H_2O_2 \xrightarrow{\text{tia } \gamma} 2{}^\bullet OH \quad (1.2)$$

$$e^-_{aq} + H_2O_2 \rightarrow {}^\bullet OH + OH^- \quad (1.3)$$

$$H^\bullet + H_2O_2 \rightarrow H_2O + {}^\bullet OH \quad (1.4)$$

The free radicals •OH generated to cause an oxidative hydrolysis reaction that scissored the β-1,4-glycoside bonds of CTS to form OC. Combining gamma-ray irradiation with low concentration H_2O_2 to produce OC has been considered a significantly scientific and practical process because it reduces requirement-radiation dose, contributing to reduced product costs.

1.4. Chitinase enzyme in plant disease prevention system

Chitinase (EC 3.2.1.14) catalyzes the hydrolysis of chitin, chitosan, peptidoglycan, and lipochitooligosaccharide, which are typical components of microbial cell membranes. Chitinase plays a role in protecting or enhancing disease resistance in plants and higher animals that do not contain chitin. Chitinase from plants has been hydrolyzing microbial cells and destroying pathogens. Concurrently, the fragments of chitin or OC molecules released from fungal cells continually cause systemic acquired resistance (SAR). Therefore, when treated with chitin or OC, plants will recognize it as being attacked by pathogens. Then, receiving and transmitting signals causes a response to activate the natural immune system or biosynthesize pathogenesis-related protein (PR-protein) to eliminate pathogens.

1.5. Soybean and anthracnose caused by the *Colletotrichum truncatum*

Soybean *(Glycine max*) is one of the most economically valuable crops and a predominant source of protein and vegetable oil for the world. They are also a pivotal industrial and food crop in the crop structure in Vietnam.

Soybean productivity is affected by many factors, of which the most common ones are diseases caused by microorganisms such as root rot, seed rot, brown spot, downy mildew, rust, anthracnose, etc. Among them, anthracnose caused by the fungus *C. truncatum* is one of the common diseases and causes heavy damage to yield (reduced to 30% – 70%). To date, the measures to control anthracnose in soybeans have mainly used chemical fungicides that may pose risks to human health and ecology. Thus, the application of new materials of natural origin to effectively control anthracnose disease on soybean plants is necessary. Several studies on using CTS, OC, or their derivatives to prevent diseases and stimulate growth in soybeans have been published. Prapagdee *et al.* (2007) used CTS to reduce the disease severity of rapid mortality disease caused by *Fusarium solani* fungus on soybean plants. Hirano *et al.* (2001) reported that using OC

increased the amount of chitinase enzyme, helping to enhance the ability to fight pathogens harmful to soybean plants. There has been no published on the disease prevention effectiveness of OC-Zn^{2+} complex on soybeans so far.

Chapter 2. MATERIALS AND METHODOLOGY

2.1. Raw materials and chemicals

Chitosan with Mw ~ 171 kDa, potato dextrose agar (PDA) fungus culture medium, $Zn(NO_3)_2{\cdot}6H_2O$, H_2O_2 30%, $C_8H_{15}O_6$, $C_7H_4N_2O_7$,... variety soybean of HL 07-15, and fungus strain of *Colletotrichum truncatum*.

2.2. Experiment and research methods

2.2.1. Preparation of OC, OC-Zn^{2+} complex, and their characteristics

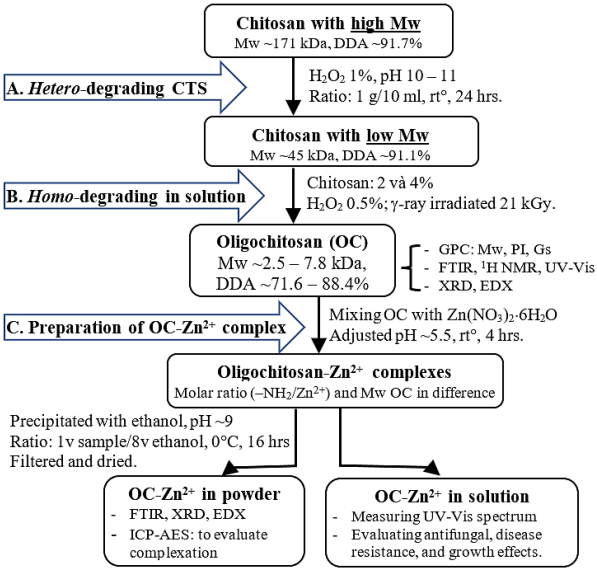

***Figure 2.1*:** Diagram of preparing OC, OC-Zn^{2+}, and characterizing properties

The approach of manufacturing OC, OC-Zn^{2+} complex, and determining the characteristic properties was according to the diagram in Figure 2.1. Chitosan powder (CTS) soaked in 1% H_2O_2 solution under room temperature, with the ratio of 1 g CTS/10 ml solution, adjusted to pH ~10 –

11 with 2M NaOH. After 24 hrs of soaking, the CTS suspension was conducted by filtering and washing with water to pH ~7 and drying to obtain low Mw CTS. Use this CTS sample to make solutions of 4% and 2% CTS in 2% & 1% lactic acid with and without the addition of 0.5% H_2O_2. Irradiation of CTS solutions was performed on a Co-60 gamma-ray source, dose rate ~1.1 kGy/h at VINAGAMMA Center to prepare OC with different Mw.

The OC-Zn^{2+} complexes in solution were prepared by mixing $Zn(NO_3)_2$ salt into OC solutions with different mole fraction ratios of ($–NH_2/Zn^{2+}$).

2.2.2. Methods and techniques used for research

UV-Vis spectroscopy: Samples were diluted with water to 0.1% OC and measured on a UV-Vis machine with wavelengths of 200 – 800 nm.

Gel permeation chromatography (GPC): The GPC graphs of CTS or OC samples were performed on an HPLC, LC–20AB Shimadzu, detector RID–10A and Ultrahydrogel 250 Waters column. Use pullulan as standard with the known Mw of 730 – 380,000 Da.

Fourier Transformed Infrared Spectroscopy (FTIR): 3 – 5 mg of sample was mixed with 200 mg of KBr, pelleted, and measured on an FTIR 8400S in the wavenumber range of 800 – 4000 cm^{-1}.

Nuclear Magnetic Resonance Proton Spectroscopy (^1H NMR): 50 mg of OC powder was dissolved in 0.5 ml of 2% CD_3COOD/D_2O solution and measured on a Bruker AVANCE III 500 spectrometer, Brucker BioSpin AG.

X-ray Diffractometry (XRD): The fine-grained powder samples of CTS, OC, and OC-Zn^{2+} complexes were pressed into film form and measured on a Siemens D5000 XRD machine, using a Cu-Kα X-ray tube (1.54 Å), voltage 40 kV, current intensity 40 mA, scanning angle 2θ in the range of 0 – 80°.

Energy-dispersive X-ray spectroscopy (EDX): Samples of OC powder and OC-Zn^{2+} complexes were compressed into film form and analyzed on an EDX, JEOL 6610 LA machine attached to the FE-SEM, S-4800, Hitachi system, recording data in the voltage range of 0 – 20 keV.

Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES): Digested OC-Zn^{2+} samples with HNO_3, and the measurements were conducted on an ICP-AES, PerkinElmer, Optima 5300DV.

2.2.3. Determination of the inhibitory effect of *C. truncatum* in *in vitro*

The *in vitro* antifungal efficiency (AE) of the materials on *C. truncatum* was assessed by the poisoned medium technique. AE% was calculated as follows: **AE (%) = (Dc – Ds) ×100/Dc**, where Dc and Ds are the fungal colony diameter (mm) of the control and test samples, respectively.

2.2.4. The effectiveness assay of anthracnose prevention and growth parameters of OC-Zn^{2+} complex on soybean plants in greenhouses

Nine seeds of the soybean (HL 07-15), treated sterilizing surface, were sown into a plastic pot 30 cm in diameter containing organic substrates. One week after sowing, pruning, and detaining four plants/pots with uniform height and size. The greenhouse experiment was arranged in a completely randomized design (CRD) with three replications.

Soybean plants were foliar sprayed with sample solutions, including zinc nitrate salt, OC, and OC-Zn^{2+} complex diluted with water to the established concentration. Foliar spraying of the test substrates thrice on the 15^{th}, 25^{th}, and 35^{th} days after sowing with 0.5, 0.75, and 1 liter/treatment. 24 hrs after the third spraying, spraying with *C. truncatum* fungal solution (density ~ 10^5 spores/ml) with a spray volume of 1 liter/treatment to infect anthracnose for soybean plants. Proceed to determination of growth parameters at 36^{th} day after sowing, chitinase enzyme activity before and after fungus spraying, and the disease severity and index at 21^{st} day after fungus spray.

The total chlorophyll content of soybean leaves 36 days after sowing was determined using the method of Arnon - 1949. The fresh leaf samples were crushed and extracted with a solvent of 80% acetol and centrifuged to collect the extracts. The optical density of the extracts was measured on a UV-Vis machine at wavelengths of 663 nm (characteristic of chl. *a*) and 645 nm

(characteristic of chl. *b*). **Total chlorophyll content (mg/g leaf) = [20.2 × (ΔA_{645}) – 8.02 × (ΔA_{663})] ×V/(1000 × W)**, where ΔA is the optical absorbance at the corresponding wavelengths; V (ml) is the volume of extracted solution; W (g) is the weight of fresh leaf tissue used for extraction.

The height of soybean plants 36th day after sowing was determined using a tape measure with 1 mm divisions, measured for 5 plants per treatment.

The dry matter content/soybean plant 36 days after sowing was assessed by cutting off the roots, drying them in an oven at 65°C to a constant weight, and weighing and calculating the dry matter content (g/plant).

Chitinase enzyme activity (CA) was determined with measuring the amount of GluNAc released from the chitin hydrolysis reaction.

Solutions of GluNAc standard at concentrations of 0.1 – 1.0 µmol/ml in phosphate buffer were used to create a standard curve showing the dependence of absorption intensity on the amount of reducing sugar GluNAc. Crude chitinase extract from soybean leaves was extracted with 0.1 M phosphate buffer (pH 7). Chitin suspension (10 mg/ml in phosphate buffer, pH 7) was used as the substrate of enzyme reaction. The reaction mixture consisted of 2 ml of chitin suspension and 2 ml of crude chitinase enzyme. After incubation for 3 hrs at 45°C, stop the reaction by boiling for 5 min, then add 2 ml of 2M NaOH solution. The reaction solution mixture (6 ml) was divided into 02 test tubes. Each tube was then supplemented with 1 ml DNS, boiled in a water bath for 5 min, and cooled to room temperature (~30°C). The absorption intensity at wavelength 540 nm was measured on a UV-Vis spectrophotometer. The CA value in leaf samples was calculated according to the formula: **CA (U/g fresh leaves) = (X.k.V)/(v.t)**, where: X is the amount of GluNAc deduced from the standard curve (µmol); k is the dilution factor (g leaves/ml); V is the total volume of the reaction (ml); v is the volume of enzyme extract used (ml); t is the reaction time (min).

The disease severity and incidence were evaluated according to QCVN 01-168:2014/BNNPTNT.

2.2.5. Evaluating the disease prevention effectiveness and agronomic efficiency of OC-Zn^{2+} complex on soybean plants in experimental fields

The experiment was arranged in a randomized complete block design (RCBD), each trial consisted of 3 treatments with 3 replications. The experimental plot area was 30 m^2/plot. Chitinase activity was determined according to section 2.2.4. Investigation of diseases and agronomic parameters according to QCVN 01-168:2014/BNNPTNT.

2.2.6. Statistical analysis of data

The data obtained from the experiments were processed by analysis of variance (ANOVA) and LSD range test at a 95% confidence level ($p < 0.05$) using MSTATC software, version 1.2, Michigan State University, USA.

Chapter 3. RESULTS AND DISCUSSION

3.1. Preparation of OC 2 – 8 kDa, OC-Zn^{2+} complex, and characteristics

3.1.1. Hetero-degraded oxidation to reduce chitosan molecular weight

1% H_2O_2 aqueous solution was used to oxidize the hetero-degrading CTS in a powder flake. After 24 hrs of reaction, the initial Mw of CTS of ~171 kDa with a polydispersity index (PI) of 3.26 was reduced to ~45 kDa with a PI of 2.78 as determined from the GPC graphs. The gained results proved that the degradation-oxidized CTS had a more uniform Mw. FTIR spectra of CTS and oxidized CTS were almost no different, proving that the degradation-reaction condition did not change the chemical structure of CTS.

3.1.2. Homo-degrading CTS in solution by gamma-ray irradiation combined with H_2O_2 to produce OC

The CTS solutions for irradiating were prepared from a ~45 kDa CTS. The reduction in Mw of CTS depended on irradiation dose and with or without adding H_2O_2 0.5% is shown in Figure 3.1. The results in Figure 3.1a show that the Mw of all three CTS samples was decreased with increasing irradiation doses. The Mw reduction extent quickly occurred at doses < 10.5

kGy, whereas at doses more than 10.5 kGy, the reduction was slowed down. At a dose of 21 kGy, the Mw of 4% CTS, 4% CTS + 0.5% H_2O_2, and 2% CTS + 0.5% H_2O_2 samples were reduced to 7.8, 5.1, and 2.5 kDa, respectively. The above findings demonstrated that adding H_2O_2 can effectively decrease the required treatment dose to prepare OC.

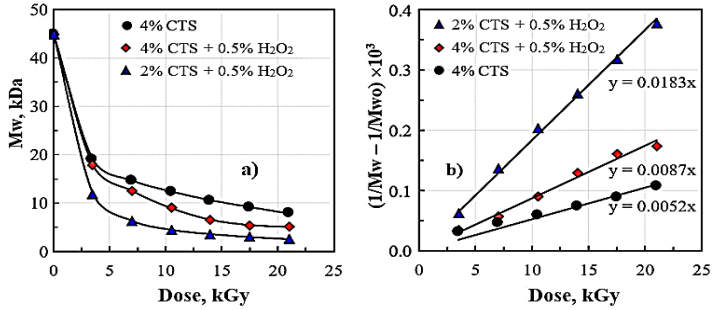

***Figure 3.1*:** The relationship of Mw, and 1/Mw – 1/Mwo with irradiation doses

From the relationship of (1/Mw – 1/Mwo) on doses (Figure 3.1b), the radiation degradation yields (Gs) were determined to be 0.4, 0.67, and 0.72 µmol/J corresponds to the irradiated chitosan solutions of 4% CTS, 4% CTS + 0.5% H_2O_2, and 2% CTS + 0.5% H_2O_2. The Gs values obtained in this study are higher than that of several earlier publications, perhaps due to the usage of CTS with high Mw in their.

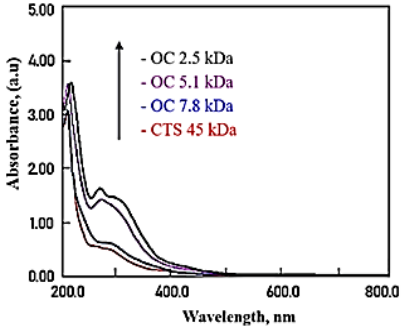

***Figure 3.2*:** UV-Vis spectra of CTS 45 kDa and OC with different Mw

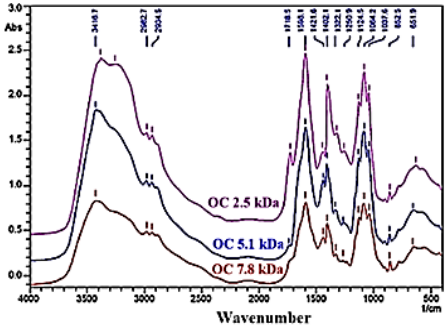

***Figure 3.3*:** FTIR spectra of OC with different Mw

The UV-Vis spectrum in Figure 3.2 shows that the initial 45 kDa CTS solution was only one absorption band at ~206 nm, which characterizes the

intermediate electronic transition in the n → σ* bond of the amido group on CTS. In the UV-Vis spectra of OCs, new peaks were observed at 265 – 281 nm, attributing to the carbonyl groups. The bands at 240 – 265 nm are assigned to the n → σ* transition of the amino groups and also the C=O specific absorption of carboxyl groups.

Figure 3.3 describes the FTIR spectra of OCs with different Mw. They all appeared the characteristic peaks of CTS: the vibrational band at 3200 – 3420 cm^{-1} corresponds to the tension vibrations of the O–H and N–H bonds, 2850 – 2900 cm^{-1} attributed the tensile vibrations of C–H, 1598 cm^{-1} characterized of $–NH_2$, 1421 cm^{-1} assigned symmetric deformation vibrations of $–CH_2$ and $–CH_3$, 1322 cm^{-1} corresponds to vibration of amide III (C–N) in $–NHCOCH_3$, 1251 cm^{-1} attributed to torsional vibration of O–H, and 1000 – 1200 cm^{-1} specified the tensile vibration of the C–O–C bond. This result proved that the chemical structure of OC was almost unchanged compared to the original CTS. However, on the FTIR spectrum of OC 2.5 kDa, a new peak at ~1718 cm^{-1} appeared with low intensity, which was characteristic of the C=O vibration of carbonyl or carboxyl. This phenomenon indicated that byproducts may be formed in the 2% CTS + 0.5% H2O2 solution irradiated with 21 kGy (OC 2.5 kDa).

The proton resonance signals of OC with different Mw are presented in Figure 3.4. The signals at 2.02 – 2.10 ppm correspond to the resonance of protons of the acetyl group ($–CH_3$) on N-acetyl-D-glucosamine units. The peaks in the region of 3.30 – 4.30 ppm are characteristic of protons H3, H4, H5, H6, and H6', and the signal at ~3.20 ppm is attributed to the H2 proton on the D-glucosamine units. Therefore, it can be seen that all the characteristic proton resonance signals of CTS appeared in the ^1H NMR spectrum of OCs. The deacetylation degree (DDA) was calculated from the ^1H NMR spectra according to the formula of Larvetu *et al.* (2003) of OC 7.8 kDa, OC 5.1 kDa, and OC 2.5 kDa reached 88.35%, 87.24%, and 71.57%, respectively. Thus, when Mw decreased, DDA values were decreased

tendentiously. However, there is no significant difference in OC 7.8 kDa and OC 5.1 kDa. This result was consistent with previously published studies.

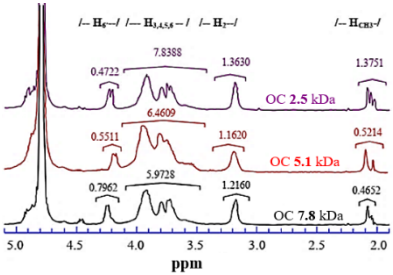

Figure 3.4: ^{1}H NMR spectra of OC with different Mw

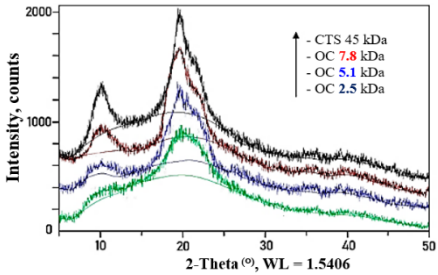

Figure 3.5: XRD patterns oa CTS 45 kDa and OC with different Mw

Using the GPC graphic analyses, the Mw and the PI of OC were determined to be Mw 7.8 kDa, 5.1 kDa, and 2.5 kDa with PI achieved respectively 1.78, 1.56, and 1.41, which indicated that the smaller the Mw OC, the lower the PI, perhaps the OC molecular chains are more uniform. The crystallinity of OCs is reflected in the XRD spectra (Figure 3.5). It observes that OC with smaller Mw have characteristic reflections at 2θ ~10.2° and ~20° that were tendentiously decreased in the intensity and expansion of the peaks. The diffraction-pattern changes manifest that irradiation-degraded CTS is reduced Mw and accompanied by a decrease in hydrogen bond density, leading to a lower crystallinity and concurrent increase in amorphous structure in the OC products.

3.1.3. Preparation and characterization of OC-Zn^{2+} complex

OC-Zn^{2+} complexes were prepared by mixing $Zn(NO_3)_2$ salt into OC solutions with pH adjusted to 5.5.

$Zn(NO_3)_2$ does not have a characteristic UV-Vis absorption peak. Meanwhile, the UV-Vis spectrum results of the OC-Zn^{2+} complex in Figure 3.6 show that the maximum absorption peak of OCs in the range of 207 – 211 nm has shifted to a larger wavelength, particularly appearing at 230.5, 227.0, and 219.5 nm for OC-Zn^{2+} complexes with OC Mw of 2.5, 5.1, and 7.8 kDa, respectively. The adsorption intensity at these peaks is higher than

that of OC due to the electron transition n - π*, π - π*, d-d, and the σ orbital of the CTS or OC ligands with the 3d orbital of the chelate ions (Cu^{2+}, Zn^{2+}).

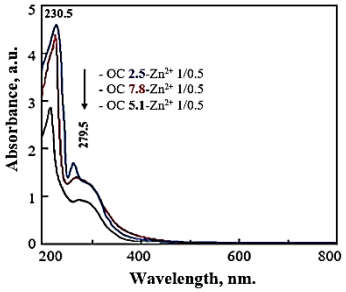

Figure 3.6: UV-Vis spectra of OC-Zn^{2+} complexes, OC with different Mw, the same molar ratio of 1/0.5

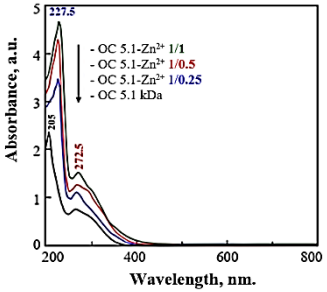

Figure 3.7: UV-Vis spectra of OC-Zn^{2+} complexes, OC 5.1 kDa, with different molar ratios

In particular, for the OC2.5-Zn^{2+} complex from OC 2.5 kDa, only one peak with high intensity at 265 nm appeared and is different from other complexes. This absorption pattern is attributed to a structural characteristic in the pendant model, which has less stability than the bridge-model structure. When the Zn^{2+} content in the OC5.1-Zn^{2+} complex increased with changing the molar ratio $–NH_2/Zn^{2+}$ from 1/0.25 – 1/1, the UV-Vis spectrum results in Figure 3.7 show that an additional small peak appears at 280 – 295 nm for complexes with molar ratios 1/0.5 and 1/1. This peak is assigned the complex with a stable bridge-model structure. Thus, OC-Zn^{2+} complexes from OC with Mw > 5.1 kDa and molar ratio from 1/0.5 – 1/1 will preferentially form complexes with a stable bridge-model structure.

FTIR spectrum analysis of OC-Zn^{2+} complexes formed from OC with different Mw showed that the characteristic peaks of OC remained, and several changes occurred concurrently (Figure 3.8). The new one at 3220 cm^{-1} may be due to the splitting vibrational of –OH, overlapping the stretching vibration of –NH, and the characteristic peak of $–NH_2$ groups at 1598 cm^{-1} on OC was shifted to a larger wavenumber at ~1602 cm^{-1}. Two other new peaks at 536 cm^{-1} and 478 cm^{-1}, believed to be the characteristic vibrations of the N–Zn and O–Zn bonds, also occurred. In addition, the

infrared absorption peak at ~1718 cm^{-1} attributed vibrations of C=O in the carboxyl, or the carboxyl on the OC molecule, has completely disappeared.

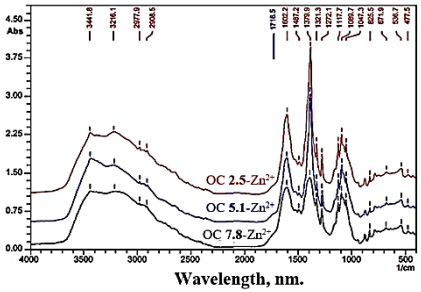

Figure 3.8: FTIR spectra of OC-Zn^{2+} complexes, OC with different Mw, the same molar ratio of 1/0.5

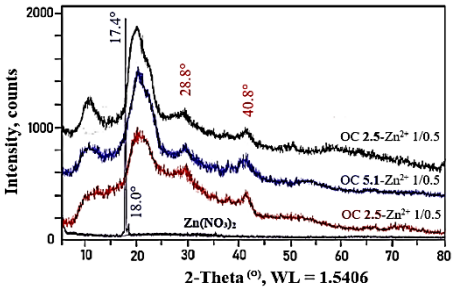

Figure 3.9: XRD patterns of $Zn(NO_3)_2$ and OC-Zn^{2+} formed OC with different Mw, the same molar ratio 1/0.5

From the above changes, it is shown that the Zn^{2+} ion formed a complex with OC through coordinating bonds with the amino groups (–NH_2), hydroxyl (–OH), and C=O of carboxyl (–COOH) or carbonyl (–COH) on the OC molecules (Figure 3.10). The coordinating bond of Zn^{2+} with the C=O bonds of carboxyl or carbonyl in the radiation-degraded OC can be considered a new finding in the thesis results.

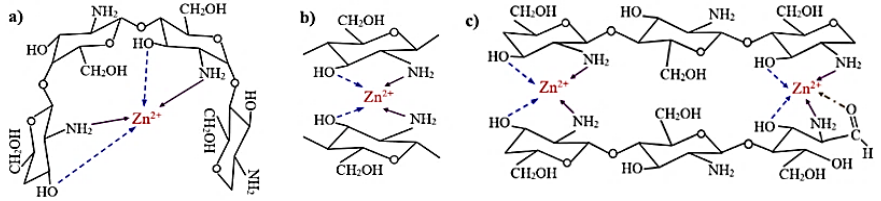

Figure 3.10: Model of the possible coordination structures of the OC-Zn^{2+} complex in bridge-pattern

The OC-Zn^{2+} complexation efficiency also depended on the Mw of OC and the mole fraction ratio (–NH_2/Zn^{2+}). Results of analysis of zinc content in complexes precipitated with ethanol using EDX and ICP-AES techniques showed that OC2.5-Zn^{2+} had a lower complexation efficiency of reaching only 88.1% in comparison with OC5.1-Zn^{2+} and OC7.8-Zn^{2+} both achieved a 90.1 – 94.4%, and the trend increases with increasing molar amount of

Zn^{2+}. The analyzed results proved that the complexation efficiency was decreased and preferentially created a less stable pendant-model complex structure in the case of Mw of OC or content of Zn^{2+} too low.

The results of the XRD patterns in Figure 3.9 show that $Zn(NO_3)_2$ appears with two sharp peaks at the 2θ of 17.4° and 18.0°, characterizing the monoclinic crystal of zinc nitrate (COD 9008175 in XRD database). For the OC-Zn^{2+} complex, two new peaks appeared at 2θ ~28.8° and 40.8°, and the peak intensity at the region 2θ ~10.0° and 20.4° was decreased compared to the corresponding OC. Thus, the interaction between Zn^{2+} and OC in the complexation reaction has led to the formation of new crystalline phases and reduced the 2θ peak intensity ~10.0°, or reduced the crystallinity of OC. The results obtained are similar to many previously published works.

3.2. Antifungal efficiency against *C. truncatum* of OC5.1-Zn^{2+} in *in vitro*

Table 3.1: Antifungal efficiency (AE%) against *C. truncatum* according to concentration of OC 5.1 kDa, Zn^{2+}, and OC5.1-Zn^{2+} with different mole fractions

Treatment	**Control (0** ppm)	**250** ppm		**500** ppm		**1000** ppm	
	D, mm	D, mm	AE, %	D, mm	AE, %	D, mm	AE, %
OC 5.1	75.7 ± 2.1	75.3^{a} ± 1.5	0.4	74.7^{a} ± 1.5	1.3	67.0^{a} ± 1.7	11.5
Zn^{2+}		–	–	–	–	44.3^{b} ± 1.5	41.4
OC-Zn^{2+} 1/0.25		61.7^{b} ± 2.1	18.5	56.7^{b} ± 1.5	25.1	18.7^{c} ± 2.5	75.3
OC-Zn^{2+} 1/0.5		58.7^{b} ± 1.2	22.5	54.3^{b} ± 2.1	28.2	06.3^{d} ± 1.5	91.6
OC-Zn^{2+} 1/1		52.3^{c} ± 2.5	30.4	09.3^{c} ± 2.5	87.7	<5	~100
CV%		3.05		4.02		5.49	
$LSD_{0.50}$		*3.56*		*3.69*		*3.52*	

** The mean values in the same column with the same letter are not statistically different, with a confidence level of $p < 0.05$.*

Antifungal efficiency against *C. truncatum* fungus causing anthracnose

on soybean plants of OC 5.1 kDa and OC5.1-Zn^{2+} complex with different concentrations of 250, 500, and 1,000 ppm, and $Zn(NO_3)_2 \cdot 6H_2O$ of 390 mg/l (equivalent to the amount of Zn^{2+} in the OC5.1-Zn^{2+} complex, molar ratio 1/0.25 after dilution to a concentration of 1,000 ppm OC) in the fungal culture PDA medium is presented in Table 3.1.

The obtained results indicated that the antifungal activity increases with concentration, and the higher the zinc content in the complex, the stronger the antifungal effect. Furthermore, the AE values reached 11.6%, 44.1%, and 75.1% corresponds to OC 5.1 kDa, Zn^{2+}, and OC5.1-Zn^{2+} complex with the mole ratio 1/0.25. Thus, the AE% value of the OC-Zn^{2+} complex is higher than the total AE% value of OC and Zn^{2+}, concluding that the OC-Zn^{2+} complex exhibited a synergistic effect in inhibitory of *C. truncatum*.

3.3. Bioactivities of OC-Zn^{2+} complex on soybean grown in greenhouses

3.3.1. Effectiveness of chitinase induction, growth stimulation, and anthracnose control on soybean of OC-Zn^{2+} with OC of different Mw

The gained results of applying OC-Zn^{2+} complexes with OC of different Mw, the same molar ratio of 1/0.5 for soybean plants showed that growth parameters such as total chlorophyll, plant height, and dry matter content were all significantly higher than those of the control (water spraying), but there was no significant difference between them. In two samples of OC2.5-Zn^{2+} complex and OC5.1-Zn^{2+} complex, the determined growth parameters in the correspondence are 2.53 mg/g fresh leaves, 43.8 cm, 5.77 g/tree and 2.32 mg/g fresh leaves, 42.3 cm, 5.86 g/tree. For the OC7.8-Zn^{2+} complex, the corresponding result reached 2.15 mg/g of fresh leaves, 40.8 cm, and 5.42 g/plant are lower than those of other complexes, but there is no statistical difference. Hence, choosing the OC-Zn^{2+} complex from OC Mw 5.1 kDa to apply in soybean growth is appropriate.

The results in Figure 3.11 show that chitinase enzyme activity (CA) in soybean leaves after inoculating infection in treatments applied with

different OC-Zn^{2+} complexes were significantly higher than that of the control + (sprayed water and sprayed pathogens). The OC5.1-Zn^{2+} complex and OC2.5-Zn^{2+} complex stimulated CA of 12.04 and 11.22 mU/g of fresh leaves, respectively, which are significantly higher than the CA of OC7.8-Zn^{2+} complex (8.43 mU/g) and the control + (2.79 mU/g) on the 3rd day after spraying the pathogen. The observed results substantiated that the chitinase stimulating efficiency on soybeans of OC-Zn^{2+} complexes has depended on the Mw of OC. The lower the Mw, the higher the CA stimulating effect.

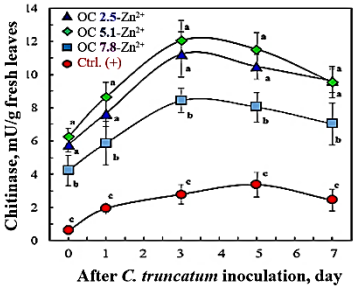

Figure 3.11: CA at different time points after *C. truncatum* inoculated on soybeans that were previously sprayed with OC-Zn^{2+} complexes of OC with Mw in difference, the same molar ratio of 1/0.5.

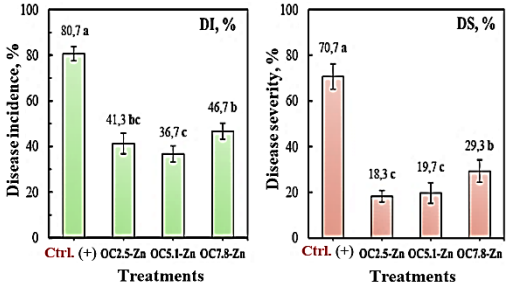

Figure 3.12: Disease incidence (DI%) and disease severity (DS%) of anthracnose on the 21st day after *C. truncatum* inoculated on soybean plants that were previously sprayed with OC-Zn^{2+} complexes of OC with Mw in difference, the same molar ratio of 1/0.5.

The results of anthracnose controllability through the ability to reduce disease incidence (DI) and disease severity (DS) are shown in Figure 3.12. The achieved values of DI are 41.3% and 36.7%, DS are 18.3% and 19.7% for OC2.5-Zn^{2+} and OC5.1-Zn^{2+} complex, respectively, which are lower than with OC7.8-Zn^{2+} complex (DI 46.7% and DS 29.3%). The high effectiveness of the OC-Zn^{2+} complex in preventing anthracnose in soybeans may be due to the direct effect of causing fungicide poisoning and the indirect pathways of stimulating soybean plants to biosynthesize the enzyme chitinase, causing decomposition and eliminating fungal invasion. From the above results, using the OC-Zn^{2+} complex to prevent anthracnose on soybeans, it has been shown that the complex with OC of Mw ~5.1 kDa is the most effective.

3.3.2. Effectiveness of chitinase induction, growth stimulation, and anthracnose control on soybean of OC-Zn^{2+} with different concentrations

Applying the OC5.1-Zn^{2+} complex (a molar ratio of 1/0.5) at concentrations of 25, 50, and 100 ppm showed that at 50 and 100 ppm, total chlorophyll, plant height, and dry matter content respectively reached 2.31 and 2.41 mg/g fresh leaves, 41.17 and 42.50 cm, 6.46 and 6.30 g/tree, that are higher than those of the control and sprayed with 25 ppm complex. There was no statistical difference between the two treatments applying complex concentrations of 50 and 100 ppm, so using OC5.1-Zn^{2+} complex with 50 ppm in concentration for soybean growth is appropriate.

Similarly, chitinase activity at concentrations applying OC5.1-Zn^{2+} complex at concentrations of 50 and 100 ppm reached the highest values and was statistically different compared with 25 ppm. After 3rd day of fungal infecting on soybeans, the chitinase activity of the two treatments reached 13.16 and 14.33 mU/g, respectively, compared to 8.08 mU/g in 25 ppm treatment. Thus, it can be concluded that applying OC5.1-Zn^{2+} complex with a concentration of 50 ppm achieved the highest efficiency in stimulating chitinase induction. Using OC5.1-Zn^{2+} to control soybean anthracnose has reduced disease incidence and disease severity. On the 21st day after fungal infecting, the DI values were 36.7% and 28.3%, and the DS values were 12.7% and 11.3%, respectively, for 50 ppm and 100 ppm treatments. Based on these results, it appears that the high concentrations of the OC5.1-Zn^{2+} complex made the anthracnose controllability of soybeans more effective.

3.3.3. Impact efficiency of OC-Zn^{2+} complex on chitinase induction, growth stimulation, and anthracnose controllability on soybean plants in compared with OC and Zn^{2+}

The recorded results showed that growth parameters of the treatment, applying complex were statistically significantly higher than those using OC or $Zn(NO_3)_2$. Chitinase activity after disease-infection spraying (Table 3.2)

of the OC5.1-Zn^{2+} complex treatment was also significantly higher than the others. Results after 3rd day of infection, the CA value in the treatment of applying complex was 11.56 mU/g, higher than the treatments using Zn^{2+} (2.65), OC (5.38), the control + (1.94), and control (0.76).

***Table 3.2*:** Chitinase activity (mU/g fresh leaves) at different time points after *C. truncatum*-inoculation on soybeans that were previously sprayed with test solutions

Treatment	0 day	1st day	3rd day	5th day	7th day
Ctrl. (water)	$0.65^{d} \pm 0.10$	$0.76^{d} \pm 0.08$	$0.74^{e} \pm 0.12$	$0.63^{d} \pm 0.11$	$0.67^{d} \pm 0.15$
Ctrl. (+)	$0.67^{d} \pm 0.09$	$1.94^{c} \pm 0.25$	$2.73^{d} \pm 0.36$	$3.18^{c} \pm 0.38$	$2.51^{c} \pm 0.29$
Zn^{2+}	$2.29^{c} \pm 0.54$	$2.65^{c} \pm 0.56$	$3.94^{cd} \pm 0.74$	$3.69^{c} \pm 0.51$	$2.31^{c} \pm 0.32$
OC 5.1 kDa	$3.85^{b} \pm 0.23$	$5.38^{b} \pm 0.65$	$6.69^{b} \pm 1.07$	$5.79^{b} \pm 0.67$	$5.67^{b} \pm 1.09$
OC5.1-Zn^{2+}	$6.31^{a} \pm 1.54$	$8.79^{a} \pm 1.43$	$11.56^{a} \pm 1.12$	$10.59^{a} \pm 0.95$	$9.77^{a} \pm 1.03$
CV%	16.74	15.14	13.98	12.43	14.07
$LSD_{0.05}$	*0.84*	*1.08*	*1.18*	*1.08*	*1.07*

* *The mean values in the same time with the same letter are not statistically different, with a confidence level of $p < 0.05$.* Ctrl. (+): water spraying – fungus spraying.

Furthermore, the CA value of the treatment applying the OC5.1-Zn^{2+} complex was higher than the total CA value of the two treatments using OC and Zn^{2+} separately, proving that the OC5.1-Zn^{2+} complex showed a chitinase-inducing effect in a synergistic effect. Similar to the above-studied contents, the values of DI ~ 38.7% and DS ~ 17.7% of the treatment using OC5,1-Zn^{2+} complex after 21 days of infection were both significantly lower than those using Zn^{2+} and OC with corresponding values of ~88.7% and ~74.3% for Zn^{2+}, and ~63.7% and ~47.7% for the OC 5.1 kDa.

3.4. Disease preventability and agronomic effectiveness of the OC-Zn^{2+} on soybean plants naturally cultivated in the experiment field

Based on the results of analyzing the physicochemical characteristics of OC 5.1 kDa, biological activity of OC5.1-Zn^{2+} complex (molar ratio 1/0.5), and appropriate concentration of OC5.1-Zn^{2+} complex (50 ppm) on soybean plants grown in the greenhouse. In this context, the disease preventability and agronomic effectiveness of OC5.1 and OC5.1-Zn^{2+} complex on soybean plants sprayed three times during the growing period were evaluated. The

results of chitinase activity, leaf moth incidence, and disease severity compared to the control are presented in Table 3.3.

***Table 3.3*:** Chitinase activity (CA), pest, and disease incidence on soybean plants tested on the 38th day after sowing

Treatment	**CA,** (mU/g fresh leaves)	**Leaf moths,** (%)	**Disease severity,** (level)	
			Brown spots	downy mildew
1. Ctrl. (water)	$0.88^{c} \pm 0.18$	9.43	2.67^{a}	1.63
2. OC 5.1 kDa	$4.15^{b} \pm 0.57$	8.53	2.61^{a}	1.40
3. OC5.1-Zn^{2+}	$7.93^{a} \pm 0.63$	7.67	1.17^{b}	1.10
CV%	13.45	14.22	11.58	10.07
$LSD_{0,05}$	*1.32*	*NS*	*0.68*	*NS*

** The mean values in the same column with the same letter are not statistically different; and NS: Non-significant difference at a probability level of $p < 0.05$.*

The CA in soybean leaves was highest in the treatment using OC5.1-Zn^{2+} complex, reaching 7.93 mU/g of fresh leaves, followed by the treatment using OC 5.1 kDa (4.15 mU/g), and the control treatment (0.88 mU/g). During the growth process, the soybean plants were infected with leaf moths (*Lamprosema indicata*) at a rate of 7.67 – 9.43%.

Investigation showed that diseases caused by fungi, such as brown spots (*Septoria glycines*) and downy mildew (*Peronospora manshurica*) appeared. The highest infection rate was in the control treatment, with the DS of 2.67 and 1.63 (according to the 9-level disease scale), respectively. In treatments using OC 5.1 kDa and OC5.1-Zn^{2+} complex, the disease level decreased, corresponding to brown spot and downy mildew disease being 2.61 and 1.40; 1.17 and 1.10. Proving that, due to the ability of the OC5.1-Zn^{2+} complex to directly inhibit fungal pathogens and stimulate soybean plants to produce the disease-preventing enzyme chitinase, it increases resistance to brown spots and downy mildew under the same culture conditions.

Growth parameters such as plant height, dry weight, and seed weight of soybeans when applied OC 5.1 kDa and OC5.1-Zn^{2+} complex were higher than that of the control. Typically, the mass of 1000 seeds reached the highest level of 154.8 g, and the net yield reached 2.61 tons/ha for OC5.1-Zn^{2+}

complex, which was statistically significantly higher than compared to OC5.1 (2.45 tons/ha), and the control (2.19 tons/ha). Applying OC5,1-Zn^{2+} complex on soybeans increased yield by 19.2% compared to the control. Therefore, the prepared OC5.1-Zn^{2+} complex has shown terrific potential for application as an effective disease-prevention agent in soybean cultivation.

CONCLUSIONS AND SUGGESTIONS

Conclusions

Based on the obtained research results, some main conclusions have been inferred as follows:

1. Appropriate conditions (CTS and H_2O_2 concentrations, radiation dose) were established to degrade chitosan to OC with a Mw of 2.5 – 7.8 kDa.
2. Successfully prepared OC-Zn^{2+} complexes with molar ratios ($–NH_2/Zn^{2+}$) and the OC Mw in different. The OC-Zn^{2+} complex made from OC 5.1 kDa and molar ratio 1/0.5 has a stable bridge-pattern complex structure, reaching more than 92% of Zn^{2+} coordinately bonded with OC.
3. OC-Zn^{2+} complex had an effective inhibiting efficiency on *C. truncatum* fungus, reaching 75.1 – 100% depending on the concentration and molar ratio ($–NH_2/Zn^{2+}$) in *in vitro* experiments.
4. OC-Zn^{2+} complex showed a synergistic effect of eliciting the enzyme chitinase and concurrently increasing the growth parameters of soybeans in greenhouse experiments. The OC-Zn^{2+} complex made from OC 5.1 kDa, molar ratio 1/0.5, with a foliar spray concentration of 50 ppm, achieved the highest efficiency among the surveyed treatments.
5. Results of field experiments by spraying foliar solution 50 ppm of OC5,1-Zn^{2+} complex three times during the growing period increased chitinase activity, reduced disease index, and ensured growth and increased soybean yield ~19% (2.61 tons/ha) compared to control (2.19 tons/ha).

The organic-inorganic hybrid OC-Zn^{2+} complex has effectively prevented

diseases and increased soybean productivity, so it has the potential for application in safe agricultural cultivation.

Suggestions

To be able to develop practical applications of OC-Zn^{2+} complex to control diseases on soybeans, some contents that need to be further performed are recommended as follows:

1. Research to evaluate the effectiveness of OC-Zn^{2+} complex in stimulating resistance and controlling some other diseases on soybean plants.
2. Coordinating with potential units to organize production, deploy large-scale testing, conform to standards and regulations, carry out marketing licensing procedures, and commercialize products to contribute to developing agricultural production in a safe and sustainable.

NEW CONTRIBUTIONS OF THE DISSERTATION

1. Successfully prepared OC-Zn^{2+} complexes with different mole fraction ratios ($–NH_2/Zn^{2+}$) and OC Mw and determined some of their properties. The OC-Zn^{2+} complex from OC 5.1 kDa with the mole fraction ratio of 1/0.5 has a complex structure mainly in a stable bridge pattern, reaching more than 92% of Zn^{2+} coordinately bonded with OC.
2. It systematically evaluated that the OC-Zn^{2+} complex has effectively prevented the fungus *C. truncatum* from causing anthracnose, eliciting enzyme chitinase, improving growth parameters, and increasing yield in soybean plants. The biostimulant effectiveness of the complex depends on the Mw of OC and the concentration of the OC-Zn^{2+} complex applied.

LIST OF THE PUBLICATIONS RELATED TO THE DISSERTATION

1. Dang Van Phu, Bui Duy Du, Le Nghiem Anh Tuan, Nguyen Quoc Hien*, *The study on antioxidant activity and enhancement of soybean seed yield of oligochitosan produced by gamma Co-60 irradiation*, Vietnam Journal of Biotechnology (ISSN: 1811-4989), **2017**, 15(3A), 179-184.

2. Dang Van Phu, Bui Duy Du, Le Nghiem Anh Tuan, Hoang Van Tam, Nguyen Quoc Hien*, *Preparation and foliar application of oligochitosan - nanosilica on the enhancement of soybean seed yield*, International Journal of Environment, Agriculture and Biotechnology, **2017**, 2(1), 421-428. DOI: 10.22161/ijeab/2.1.53.
3. Dang Van Phu, Bui Duy Du*, Le Nghiem Anh Tuan, Le Thanh Hung, Nguyen Quoc Hien, *Preparation of radiolysis-degraded oligochitosan, oligochitosan-Zn^{2+} complex and their induced effect against anthracnose on soybean plants*, Vietnam Journal of Chemistry (ISSN: 2525-2321), **2019**, 57(3), 363-367. DOI: 10.1002/vjch.201900045.
4. Dang Van Phu, Bui Duy Du*, Le Nghiem Anh Tuan, Le Thanh Hung, Hoang Dac Hiet, Nguyen Quoc Hien*, *Preparation and antifungal activity investigation of oligochitosan-Zn^{2+} on Colletotrichum truncatum*, International Journal of Polymer Science (ISSN: 1687-9422), **2019**, Article ID 8357381, 6 pages. DOI: 10.1155/2019/8357381. WoS (SCIE), IF: 2,792, Q2, H index: 50.
5. Phu Van Dang, Ha Thi Tran, Duy Ngoc Nguyen, Quoc Anh Le, Du Duy Bui, Hien Quoc Nguyen, Cong-Sac Tran, Ha Manh Bui, *Study on the chitinase-induced efficiency against anthracnose on soybean plant by oligochitosan-Zn^{2+} complexes*, Case Studies in Chemical and Environmental Engineering (Online ISSN: 2666-0164), **2023**, 7, Article ID 100285, 6 pages. DOI: 10.1016/j.cscee.2022.100285. Scopus, SJR: 1,26, Q1, H index: 22.
6. Dang Van Phu*, Nguyen Ngoc Duy, Le Anh Quoc, Bui Duy Du, Nguyen Quoc Hien, *Characterizations of oligochitosan-degraded irradiation and chitinase-induced efficiency by oligochitosan-Zn^{2+} complexes on soybean plant*, Proceedings of Vietnam Conference on Nuclear Science and Technology (ISBN: 978-604-67-2718-7), VINANST-15, Nha Trang, Khanh Hoa, Vietnam, **2023**, 404-410, Science and Technics Publisher.