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Induction of Anthracnose Disease Resistance on Chili Fruit by Treatment of Oligochitosan—Nanosilica Hybrid Material

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Abstract

Oligochitosan (OC) with molecular weight Mw of 5000 g/mol was prepared by gamma Co-60 ray irradiation of chitosan solution. Nanosilica ($nSiO_2$) with the size of 10 - 30 nm was synthesized by calcination of acid treated rice husk. The mixture of 1% OC - 1% $nSiO_2$ was prepared by dispersion of $nSiO_2$ in OC solution. The morphology of $nSiO_2$ in the mixture of OC-$nSiO_2$ was measured from images of transmission electron microscopy (TEM). The effect of foliar application of the mixture of OC-$nSiO_2$ on the induction of resistance against anthracnose disease caused by *Colletotrichum gloeosporioides* fungus on chili fruits was investigated. Results indicated that foliar application of OC-$nSiO_2$ with the concentration of 60 mg/l - 60 mg/l was found to be as the optimal treatment that reduced the disease severity on chili fruits to 22.2% compared with 90.0% of the control. Thus, OC-$nSiO_2$ hybrid material could be considered as an effective biotic elicitor to prevent anthracnose disease infection for chili fruits. Furthermore, the prepared OC-$nSiO_2$ hybrid material can also be used as an environmentally friendly agrochemical product for sustainable development of agriculture.

Keywords

Oligochitosan, Nanosilica, Chili Fruit, *Colletotrichum gloeosporioides*, Anthracnose

1. Introduction

Chitosan and oligochitosan (OC) have attracted considerable interest due to their many unique biological activities such as antioxidant activity [1], antimicrobial activity [2], plant growth promotion such as rice [3], coffee [4], soybean [5], ... In addition, chitosan and OC also have elicitation effect to induce disease resistance in plants such as grapevine [6], potato [7], ... Moreover, OC was supposed as a plant vaccine that is similar to general animal vaccine [8]. The excessive use of chemical fertilizer and pesticide in agriculture may lead to adverse effects in food products and in the environment. Therefore, the recent trend in agriculture has been focused on organic and vertical farming not only addressing the rising concern for environmental issues but also accommodating the demands of food of increasing world population [9]. Organic farming is considered as a viable alternative in comparison to chemical-based agriculture [10]. Nanotechnology opens up a wide applicability in various fields like medicine, pharmaceutics, electronics and agriculture. Nanomaterials hold great promise of improved plant disease resistance, controlled release of agro-chemicals, enhanced plant growth, etc. [11]. According to [12], nanomaterials can be used as a magical tool for enhancing growth and improvement of agricultural production. Recently, the synergistic effect of a mixture of chitosan-silica induced resistance in tomato against bacterial wilt caused by *Ralstonia solanacearum* was reported for the first time [13]. Chili fruit is widely consumed mainly as a spice for food ingredients. World production of chilies in 2009 was estimated to be about 3 million tones [14]. The top-ten chili producing countries were India, China, Ethiopia, Myanmar, Mexico, Vietnam, Peru, Pakistan, Ghana and Bangladesh, and accounted for more than 85% of the world chili production in 2007 [15]. Anthracnose disease has been a real threat to chili (*Capsicum* spp.) production in the tropics and subtropics worldwide. Anthracnose is caused by a complex of *Colletotrichum* species, typically *Colletotrichum capsici*, *Colletotrichum acutatum*, and *Colletotrichum gloeosporioides* having been reported as significant pathogens in many countries including China, India, Brazil, Thailand and Vietnam, etc. [16]. Therefore, protection of chili fruits from infection of anthracnose disease is in high demand. Up to now, there are rare reports in the literature on the reduction of anthracnose disease on chili fruits using OC-nSiO_2 hybrid materials.

To contribute to the development of organic farming, in the present study, OC was prepared by gamma Co-60 irradiation degradation of chitosan in solution and nSiO_2 was prepared from rice husk. The effect of foliar application of OC-nSiO_2 mixture on the induction of resistance against anthracnose disease caused by *Colletotrichum gloeosporioides* fungus on chili fruits was investigated.

2. Materials and Methods

2.1. Preparation of Oligochitosan by γ-Irradiation

Chitosan from shrimp shell with a degree of deacetylation (DDA%) of ~91.4%; the weight average molecular weight (Mw) of 44.5×10^3 g/mol was supplied by a factory in Vietnam. OC with Mw of 5000 g/mol was prepared by gamma Co-60 ray irradiation degradation method as described in our previous paper [17] with some modifications. Briefly, chitosan (40 g) was dissolved in 800 ml of 2% (w/v) lactic acid solution, then 15 ml of hydrogen peroxide (30% H_2O_2) and 185 ml water were added to prepare 1 liter of 4% (w/v) chitosan solution containing 0.5% H_2O_2. Then, the prepared chitosan solution was irradiated at ambient condition on gamma SVST Co-60/B irradiator at the VINAGAMMA Center with a dose of 21 kGy.

2.2. Preparation of Nanosilica from Rice Husk

$nSiO_2$ with particles size of 10 - 30 nm was prepared from rice husk as described in our previous paper [18]. Briefly, raw rice husk was first rinsed with water to remove dusts, soluble substances, and other contaminants. It was then dried at 60˚C in forced air oven (Yamato, DNF 410, Japan). 50 g of the dried rice husk was then treated with 500 ml of 0.5 N HCl at ambient temperature for 2 h by magnetic stirring. Then, it was decanted and thoroughly washed with distilled water until the rinse became free from acid. The treated-rice husk was subsequently dried in forced air oven until to dry and ground into fine powder. Finally, the rice husk powder was incinerated at 700˚C for 2 h inside a programmable furnace (Nabertherm GmbH, Germany) to obtain $nSiO_2$.

2.3. Preparation of Oligochitosan-Nanosilica Hybrid Material

10 g of $nSiO_2$ were mixed with 77 ml of NaOH 1M solution by string for 60 min. Then 250 ml of 4% OC solution and distilled water slowly added to prepare 1 liter of 1% OC-1% $nSiO_2$. The mixture was then stirred for 4 h at room temperature. To increase the adsorption ability of OC on $nSiO_2$, pH of the mixture was adjusted to ~7.5 [19]. The particle size of $nSiO_2$ from rice husk and $nSiO_2$ in the OC-$nSiO_2$ hybrid material was measured using transmission electron microscopy (TEM) images [18].

2.4. Preparation of *Colletotrichum gloeosporioides* Fungal Suspension

The *C. gloeosporioides* fungal strain was obtained from Microbial Laboratory of the Research and Development Center for Hi-Tech Agriculture. An inoculum of *C. gloeosporioides* was prepared by cultivating fungal colonies in Petri dishes on potato dextrose agar (PDA) medium at 30˚C for ten days. Then, the fungal spores were harvested by flooding Petri dishes with sterile distilled water. The obtained suspension of fungal spores was then adjusted to 10^4 spores/ml with distilled water [20].

2.5. Experimental Design

The 60 - 65 day-old-chili plants (*Capsicum frutescens* L.) with young green fruits were used for the study. The experiment was conducted in the greenhouse of Hi-Tech Agriculture Center, Cu Chi, Ho Chi Minh City, at 30˚C ± 2˚C and

RH of 60% ± 2%. The experimental treatments were arranged in a randomized complete block design with five treatments namely: control (water), 20 - 20, 40 - 40, 60 - 60 and 80 mg/l - 80 mg/l of OC-nSiO_2. Particularly, 1 liter of 1% OC - 1% nSiO_2 was diluted with water up to the volume of 500, 250, 167, and 125 liters for preparation of foliar spaying solution of 20 - 20, 40 - 40, 60 - 60 and 80 mg/l - 80 mg/l of OC-nSiO_2, respectively. Each treatment consisted of three replications with 10 plants/replication.

2.6. Foliar Application of OC-nSiO_2 on Chili Plants and Fungal Inoculation on Chili Fruits

The chili plants were sprayed three times with a duration of one time per week. After 24 h of the third spaying, the chili fruits were caused lesions as follows: 50 fruits/chili plant were randomly selected to create lesion by using a needle with a diameter of 0.6 mm to pierce through the fruits. Then, the injury fruits were inoculated with *C. gloeosporioides* fungus by spaying fungal spore suspension of 10^4 spores/ml.

2.7. Assessment of Disease Severity

After 7 days of fungal inoculation, the disease severity (DS) was then recorded and calculated as follows [21]:

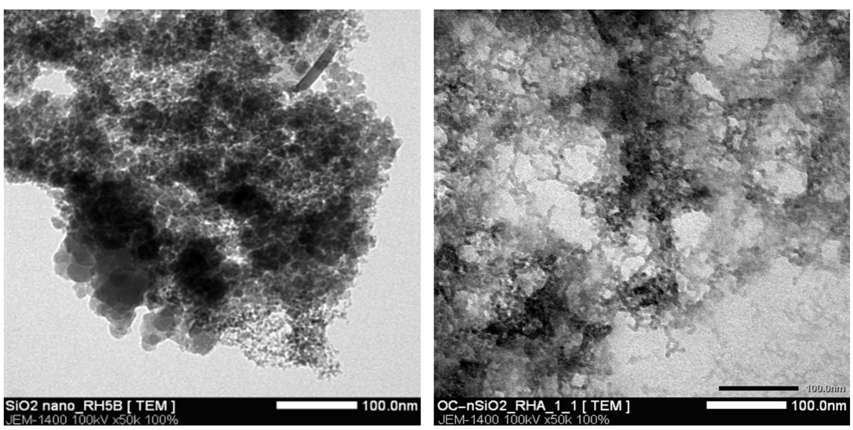
DS (%) = 100 × (number of infected fruits)/(total number of inoculated fruits)

All data were statistically analyzed by analysis of variance (ANOVA) according to the experimental design and Least Significant Difference (LSD) at 5% probability level was utilized to compare the different means.

3. Results

3.1. Morphology of nSiO_2

TEM images of nSiO_2 and nSiO_2 in the mixture of 1% OC - 1% nSiO_2 were presented in Figure 1. The size of nSiO_2 synthesized from rice husk was of 10 - 30 nm in



(a) (b)

Figure 1. TEM images of nSiO_2 (a) and nSiO_2 in mixture of 1% OC - 1% nSiO_2 (b).

Figure 1(a) and the size of $nSiO_2$ in the OC-$nSiO_2$ mixture in Figure 1(b) was observed to be rather smaller to that of original $nSiO_2$.

The schematic interaction of OC with $nSiO_2$ was illustrated in Figure 2. When $nSiO_2$ was mixed with OC solution, the bonding of −Si-O-C−, −Si-O-H_2N−, and −Si-OH occurred in the mixture of OC-$nSiO_2$.

3.2. Anthracnose Disease Severity on Chili Fruits

Photographs of chili plants in greenhouse and symptom of anthracnose disease with concave lesion stains in slightly brown color on chili fruits caused by *C. gloeosporioides* fungus were presented in Figure 3.

The disease severity of chili fruits treated with OC-$nSiO_2$ with different concentration and inoculated with *C. gloeosporioides* fungal spores was presented in Table 1.

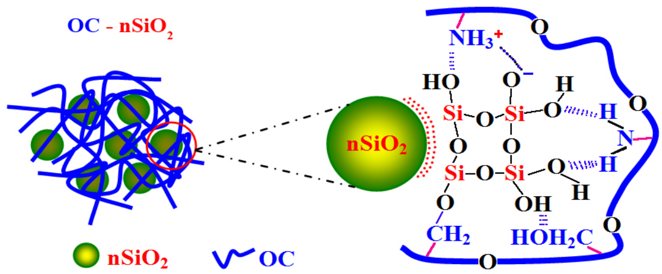


Figure 2. Schematic illustration of interactions between OC and $nSiO_2$.

(a) (b)

Figure 3. Photographs of chili plants in greenhouse (a) and symptom of anthracnose disease on chili fruit caused by *C. gloeosporioides* fungus (b).

Table 1. Disease severity of chili fruits inoculated with *C. gloeosporioides* fungal spores.

OC-$nSiO_2$, mg/L	DS, %
Control (water)	90.0 ± 11.5^{d}
20 - 20	62.2 ± 5.1^{c}
40 - 40	35.6 ± 1.9^{b}
60 - 60	22.2 ± 1.9^{a}
80 - 80	33.3 ± 8.8^{b}

Different letters in the same column indicate significant differences at $P \leq 0.05$.

4. Discussion

The greatest challenge in the application of OC for plant protection lies in the development of efficient methods for large-scale production of OC [22]. For this aspect, the production of OC by gamma Co-60 irradiation method can be favorably applied on large-scale [17] [23].

TEM image in **Figure 1(b)** indicated that the $nSiO_2$ morphology was almost maintained as the original $nSiO_2$. However, some small parts were aggregated that may be presumed due to the interaction of $nSiO_2$ with OC. Also, $nSiO_2$ may be changed to $Si(OH)_4$ in slightly basic medium pH ~7.5 [19]. As our observation, OC solution, unlike chitosan, is not precipitated in neutral and/or slightly basic medium. As can be observed in **Figure 2** that the bonding of –Si-O-C–, –Si-O-H_2N–, and –Si-OH between OC and $nSiO_2$ was formed [21] and owing to these bonds, the colloidal mixture of OC-$nSiO_2$ was in good stability during storage. Accordingly, the bonding of chitosan and $nSiO_2$ in the mixture of chitosan-$nSiO_2$ [24] was almost the same as in the mixture of OC-$nSiO_2$.

Results in **Table 1** indicated that foliar application of OC-$nSiO_2$ induced significantly anthracnose disease resistance caused by *C. gloeosporioides* fungus on chili fruits. Among four treatments with different concentration of OC-$nSiO_2$, the treatment with a concentration of 60 mg/l - 60 mg/l was found to act the highest effect in reduction of anthracnose disease on chili fruits. In particular, the disease severity of the treatment of 60 mg/l - 60 mg/l OC-$nSiO_2$ was reduced to 22.2% compared to 90.0% of the control (water). It was reported that the bacterial wilt incidence on tomato plants was also significantly reduced by the treatment with chitosan-silicon mixture [13]. In particular, the bacterial wilt incidence was of ~20% for tomato plants treated with chitosan-silicon compared to ~80% of the control (without chitosan and silicon, inoculation with *R. solanacearum*). Recently, foliar application of OC-$nSiO_2$ with a concentration of 50 mg/l - 50 mg/l increased soybean seed yield of 17% compared to control (water) [23]. Thus, it can be expected that foliar application of OC-$nSiO_2$ is not only to induce disease infection resistance but also to increase chili fruit yield. Further study on the effect of OC-$nSiO_2$ treatment on the enhancement of chili fruit yield should be carried out. Moreover, the nanomaterials, owing to their increased contact surface area, might have toxic effects that are not apparent in the bulk materials especially in the open agricultural ecosystem [24]. Therefore, the selection of nanomaterial for application in the field may be critically desirable as materials, which are non-toxic, biocompatible and biodegradable [25]. In this issue, it was reported that different form of synthetic amorphous silica has been used in a wide variety of industrial and consumer applications including food, cosmetics and pharmaceutical products for many decades, and no environmental or health risks have been associated with these materials including $nSiO_2$ [26]. Therefore, the as-prepared OC-$nSiO_2$ hybrid material can be considered as a safe product to human health and environment.

5. Conclusion

The method of gamma Co-60 ray irradiation degradation of chitosan in solution to prepare oligochitosan can be favorably applied on large scale. Nanosilica, a value-added product was appropriately synthesized from rice husk, an abundant agriculture waste. Foliar application of OC-nSiO_2 on chili plant resulted in significant induction of anthracnose disease resistance caused by *C. gloeosporioides* fungus on chili fruits. Thus, the OC-nSiO_2 hybrid material originated from natural resources can be potentially used to replace hazardous agrochemicals for sustainable agricultural production.

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