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# Research Article
# Effects of Chitosan and Silica Nanoparticles Against the Development and Growth of Red Chilli Anthracnose Disease *Colletotrichum* sp.

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## Abstract

**Background and Objective:** *Colletotrichum* sp., is a pathogen that causes anthracnose disease that can reduce chilli production. One environmentally for controlling plant disease can be done using chitosan and silica nanotechnology. This study aimed to test the ability of chitosan and silica nanoparticles to inhibit the growth of *Colletotrichum* sp. and suppress the development of the diseases on chilli seeds. **Materials and Methods:** This research consisted of the pathogenicity test of chitosan and silica nanoparticles and chilli seed germination inhibition test to control the development of *Colletotrichum* sp., using a completely randomized design within 10 treatments and 3 replications. **Results:** The results showed that 100 ppm chitosan nanoparticles inhibited the growth of *Colletotrichum* sp. and conidia germination with inhibition percentages of 92.20 and 99.4%, respectively. In addition, the development of anthracnose on chilli seed germination has been suppressed by 93% at a 100 ppm concentration of silica nanoparticles. **Conclusion:** In conclusion, both single or mixed formulations of chitosan and silica nanoparticles were able to inhibit the growth and development of *Colletotrichum* sp. and increase the chilli seed viability.

Key words: Biopesticide, silica, chitosan, anthracnose disease, *Colletotrichum* sp., nanotechnology, chilli

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

One pathogen that infects many horticultural plants is *Colletotrichum* sp., which is the cause of major diseases. Diseases caused by the fungus *Colletotrichum* sp., can infect several plants such as apples, strawberries, pears, tomatoes[1], olives[2] and coffee[3]. The chilli plant is one of the vegetables that can be attacked by this pathogen, affecting yield losses up to 50% and resulting in crop failure[4]. Damping-off on chilli seedlings which infect seeds and the decreases seed germination was caused by *Colletotrichum* sp[5].

Nanotechnology has the advantages of high efficacy and safety levels, reducing the dose or concentration of pesticide use on crops, reducing toxic residues and reducing environmental emissions on agricultural land. The development of the nanotechnology concept is a promising solution which is considered economically, safe environmentally friendly that can be implemented in any green chemistry design[6].

Chitosan nanoparticles can inhibit the growth of several fungi including *Fusarium solani* f. sp., *glycines*[7], *Botrytis cinerea*, *Penicillium expansum*[8], *Rhizopus stolonifer*[9], *Alternaria kikuchiana, Physalospora piricola*[10] and *Alternaria alternata*[11]. The provision of chitosan nanoparticles was effective in controlling *Colletotrichum gloeosporioides* that cause anthracnose in mangoes[12]. In addition, chitosan was also able to inhibit the growth of *Colletotrichum musae* in banana and dragon fruits weight lost, titratable acidity and fruit firmness[13]. Chitosan nanoparticles exceed in antimicrobial activity than the parent chitosan because of the accumulated the chitosan and tripolyphosphate molecules which gave the small size of the particle and specific high the surface energy[14].

Silica nanoparticle mixed with carbon fibre 80 mesh 5% and *T. harzianum* has an antagonistic effect on *Sclerotium rolfsii* on soybeans plants growth by 58-76-80.92%[15]. Moreover, silica nanoparticle applications directly or indirectly increased the anti-herbivore hormonal defences of the plant and attracted the predator against the pest which potentially can be vectors of pathogen viruses, thus reducing the risk of viral infections in plants[16]. Therefore, the objective of this research was to obtain the effective concentration both from single to mixed (chitosan and/or silica nanoparticles) to suppress the development of *Colletotrichum* sp., *in vitro* and to control the germination of anthracnose disease in red chilli seeds.

## MATERIALS AND METHODS

**Research site:** The research was conducted from December, 2020 to May, 2021 at the Laboratory of Phytopathology, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Padjadjaran (6°35'32.72"S and 107°64'55.73"E).

***Colletotrichum* sp. isolation:** Chilli fruit which shows symptoms of anthracnose disease was taken randomly for about 10 fruits as a representative from the research field of the Faculty of Agriculture, Universitas Padjadjaran. Chilli fruit was cleaned with running water followed by sterile water and cut in 1×1 cm. These small pieces are dipped into a 70% alcohol solution for 15 sec and then soaked in a 1% solution of sodium hypochlorite for a minute. Sterilized tissue was placed on the surface of the potato dextrose agar (PDA) medium on a petri dish and incubated at room temperature (±28°C). The growing colony is then isolated and shed on a new PDA medium for a pure culture of *Colletotrichum* sp.

**Pathogenicity test on the seeds of red chilli plants:** The chilly seeds were treated with hot water at a temperature of 50 for 25 min. Then, the seeds were soaked with a single and mixed silica and chitosan nanoparticles treatment, control treatment and pesticides made from difenoconazole for 30 min. Furthermore, the seeds are soaked into a spore suspension (with a density of 107). After 30 min in a suspension, the seeds were twisted and put on the filter paper for a few minutes. The 25 seeds were then put in a petri dish that already contains moist sterile filter paper. Seeds for non-subverted treatment of *Colletotrichum* sp. placed directly on the surface of moist sterile filter paper. Petri dishes are stored in mica plastic which already added sterile aqua dest as a basic surface to retain the moisture. The inhibition was calculated based on the results of measurements of the diameter of the fungal colony. The calculation of the diameter of the colony is done by making vertical and horizontal lines intersect precisely at the midpoint of the fungal colony on the outside of the petri dish base until full control treatment[17].

**Germination test of spore structures:** The germination test of conidia *Colletotrichum* sp., was carried out by inserting each treatment into a 5 mL reaction tube. The control treatment was added with sterile aqua dest. Next, the *Colletotrichum* sp., pieces were added by taking five pieces of pure culture aged 14 days and vortexed for 3 min. The

Table 1: Formulation of chitosan and silica nanoparticles for this research

| Codes | Treatments |
|---|---|
| A | Control |
| B | Chitosan nano 50 ppm |
| C | Chitosan nano 100 ppm |
| D | Chitosan nano 200 ppm |
| E | Silica nano 50 ppm |
| F | Silica nano 100 ppm |
| G | Silica nano 200 ppm |
| H | Chitosan nano 50 ppm+silica nano 50 ppm |
| I | Chitosan nano 100 ppm+silica nano 100 ppm |
| J | Fungicide (difenoconazole 250 gr) |

suspension on the reaction tube was then taken using a micropipette by 1 mL into a watch glass, incubated in a sterile box for 24 hrs and then observed at 400× magnification. Observations are made on the number of conidia germinated. The percentage of germination of conidia (GC) is calculated using the following formula[18]:

$$GC\ (\%) = (\Sigma\ \text{Germinates conidium}: \Sigma\ \text{Observed conidium}) \times 100$$

In addition, observations were made on the suppression test of the disease of sprouts, including the growth of sprouts (%), height and length of the roots. Observation of the length of the roots is carried out by taking 1 sprout of 25 sprouts on each petri dish, daily until the age of 21 days.

**Data analysis:** The treatments were done with 10 treatments (Table 1) and 3 replications using Completely Randomized Design (CRD). Data experiments were analyzed using the variance method (ANOVA) from the IBM SPSS® software ver. 24.0 for Windows. When requirements for normality of data and homogeneity of variance were satisfied, the analysis was assessed at a 95% confidence level to compare the differences between treatment means.

## RESULTS AND DISCUSSION

**Inhibition of the formulation on the growth of *Colletotrichum* sp.:** The nano chitosan and silica inhibited the growth of *Colletotrichum* sp., colonies in almost all the treatments tested (Table 2). Diameter colony *Colletotrichum* sp., of all treatments differ markedly from control treatments, except nano-silica 100 and 200 ppm. Based on the observations for 12 days showed that the growth of *Colletotrichum* sp., fungus on nano chitosan was 100 ppm, slower than the controls on PDA media. Chitosan as a reducing agent, in its development, has antimicrobial properties that are formulated with Cu which has anti-fungal properties and is not toxic to living things[19].

Chitosan nano concentrations of 100 and 200 ppm have markedly different inhibitory results because the higher the chitosan concentration used, the inhibition of pathogen growth decreases. This phenomenon is thought to be too many chitosan molecules, then reactive amino groups will also be countless so that the ability of chitosan to stick to the surface of pathogens decreases[20]. Chitosan nano can inhibit the growth of *Colletotrichum* sp., due to the chitosan content that can cause impaired cell permeability that triggers cell death with its main constituent being chitin[21]. Growth inhibition mechanism *Colletotrichum* sp., involves chitosan interaction with the cell membrane of the pathogen[19]. Through electrostatic interactions, chitosan polycation is attached to the negative charge of the cell membrane which exerts influence on the permeability and intracellular leakage[19].

The antifungal effect of nanosized silica-silver was also studied on papaya, mango and orange which showed the silicon treatment consistently reduced the anthracnose disease by *Colletotrichum*[22,23]. This could happen because silica has antifungal properties that can inhibit the growth of *Colletotrichum* sp., causes anthracnose. It is also believed that Si obtains the indirect mode of action in plant biochemical defence which affects the protection of mechanical and physical cell walls from pathogens penetration[22,24].

The concentration of single nano chitosan and silica compared to the mixture does not differ markedly when compared to the 100 ppm chitosan nano treatment which has an inhibition percentage of 92.20%. However, if the mixture is compared to a single nano-silica of 200 ppm, the result showed that does not differ markedly. This happened because the greater the concentration of nano-silica used in the formulation, the greater the width of the resulting inhibitory zone.

**Effect on the germination of *Colletotrichum* sp. conidia:** *Colletotrichum* sp., which have been incubated for 24 hrs showed that all treatments are different from control treatments from their conidia (Fig. 1a-b). As seen in Table 3, the obtained result tends to be higher the concentration of nano chitosan than the germination of the higher the conidia. However, the single nano-silica used the higher the concentration, the lower the germination was. The higher the concentration of chitosan, the growth inhibitory of pathogens decreases. This is evident in the single chitosan nano-treatment that shows the average germination of *Colletotrichum* sp., mushroom conidia. This supports other studies that the greater the concentration of nano chitosan

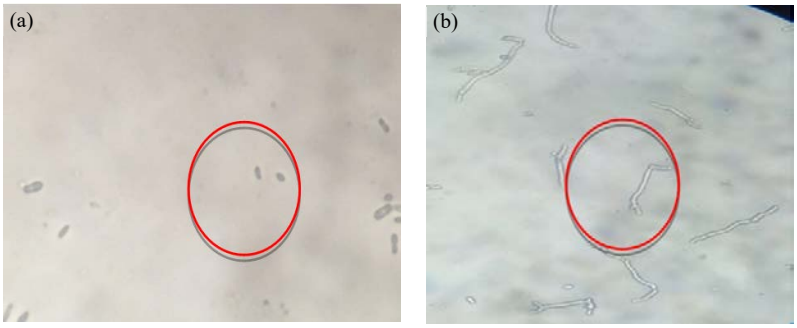


Fig. 1(a-b): Conidia of *Colletotrichum* sp., (a) Ungerminated conidia and (b) Germinating conidia

Table 2: Diameter of the colony dan percentage inhibition of the growth of *Colletotrichum* sp. in various treatments

| Codes | Treatments | Diameter of colony (cm) | Inhibition of growth (%) |
|---|---|---|---|
| A | Control | $9^{c}$ | 0 |
| B | Chitosan nano 50 ppm | $5^{b}$ | 44.4 |
| C | Chitosan nano 100 ppm | $0.8^{a}$ | 92.20 |
| D | Chitosan nano 200 ppm | $7^{bc}$ | 15.50 |
| E | Silica nano 50 ppm | $4.3^{b}$ | 52.2 |
| F | Silica nano 100 ppm | $7^{bc}$ | 22.22 |
| G | Silica nano 200 ppm | $6.3^{bc}$ | 55.55 |
| H | Chitosan nano 50 ppm+silica nano 50 ppm | $4.6^{b}$ | 60 |
| I | Chitosan nano 100 ppm+silica nano 100 ppm | $4^{b}$ | 55.55 |
| J | Fungicide | $0.54^{a}$ | 94.44 |

Same letter on one column in the table shows the data is not significantly different from the controls based on Duncan's 5% multiple distance test

Table 3: Percentage of germination and inhibition growth of conidia *Colletotrichum* sp.

| Codes | Treatments | Conidia germination | Inhibition of conidia (%) |
|---|---|---|---|
| A | Control | $17^{d}$ | 0 |
| B | Chitosan nano 50 ppm | $0.1^{a}$ | 99.4 |
| C | Chitosan nano 100 ppm | $0.5^{a}$ | 97 |
| D | Chitosan nano 200 ppm | $0.5^{a}$ | 97 |
| E | Silica nano 50 ppm | $9.7^{c}$ | 42.9 |
| F | Silica nano 100 ppm | $9.7^{c}$ | 42.9 |
| G | Silica nano 200 ppm | $6.3^{bc}$ | 62.9 |
| H | Chitosan nano 50 ppm+silica nano 50 ppm | $0.2^{a}$ | 98.8 |
| I | Chitosan nano 100 ppm+silica nano 100 ppm | $2^{a}$ | 88.2 |
| J | Fungicide | $3^{ab}$ | 82.3 |

Same letter on one column in the table shows the data is not significantly different from the controls based on Duncan's 5% multiple distance test

contained in the fungal growth medium, the more the amount of solution that diffuses into the fungal cells will cause inhibition of fungal growth which causes the death of the fungus[25].

**Incubation period of anthracnose disease on chilli seeds:** Based on Table 4, soaking chilli seeds with nano chitosan and single silica and mixed has a real effect in suppressing the incidence of sprout blight disease except for 50 ppm nano-silica treatment and fungicide treatment. The lowest incidence of the disease is found in the 100 ppm nano silica treatment with a percentage of 1.3%. Giving Si to plants can improve physiological functions, strengthen tissues and increase plant resistance to pests thereby increasing plant growth and production[16].

**Occurrence and suppression of anthracnose disease on chilli seed:** The results of statistical analysis data on the incidence of germinating seedling disease in Table 5 showed that seeds given the nano-silica treatment of 100 ppm were affected compared to control. Meanwhile, the treatment of chitosan nano at concentrations of 50, 100 and 200 ppm was significantly different from the treatment of a mixture of nano-silica and chitosan at 50 and 100 ppm. Compared to nano-silica and chitosan treatments of 50 and 100 ppm, single nano-silica of 200 ppm was not significantly different. The use

Table 4: Incubation period of anthracnose disease on chilli sprouts

| Codes | Treatments | Days after inoculations | Disease incidence (%) |
|---|---|---|---|
| A | Control | 6 | 18.6[b] |
| B | Chitosan nano 50 ppm | 6 | 29.3[cde] |
| C | Chitosan nano 100 ppm | 4 | 33.3[d] |
| D | Chitosan nano 200 ppm | 6 | 32[cde] |
| E | Silica nano 50 ppm | 4 | 21[bc] |
| F | Silica nano 100 ppm | 11 | 1.3[a] |
| G | Silica nano 200 ppm | 7 | 33.3[d] |
| H | Chitosan nano 50 ppm+silica nano 50 ppm | 4 | 33.3[d] |
| I | Chitosan nano 100 ppm+silica nano 100 ppm | 4 | 38.6[d] |
| J | Fungicide | 9 | 13.3[ab] |

Same letter on one column in the table shows the data is not significantly different from the controls based on Duncan's 5% multiple distance test

Table 5: Incidence and suppression of anthracnose disease on 12 days after inoculation (DAI) chilli plants

| Codes | Treatments | Disease incidence (%) | Percentage of the suppression (%) |
|---|---|---|---|
| A | Control | 18.6[b] | 0 |
| B | Chitosan nano 50 ppm | 29.3[cde] | 63.4 |
| C | Chitosan nano 100 ppm | 33.3[d] | 55.8 |
| D | Chitosan nano 200 ppm | 32[cde] | 58.1 |
| E | Silica nano 50 ppm | 21[bc] | 88.5 |
| F | Silica nano 100 ppm | 1.3[a] | 93 |
| G | Silica nano 200 ppm | 33.3[d] | 55.8 |
| H | Chitosan nano 50 ppm+silica nano 50 ppm | 33.3[d] | 55.8 |
| I | Chitosan nano 100 ppm+silica nano 100 ppm | 38.6[d] | 48.1 |
| J | Fungicide | 13.3[ab] | 28.4 |

Same letter on one column in the table shows the data is not significantly different from the controls based on Duncan's 5% multiple distance test

Table 6: Ability of chilli sprouting at 12 days after seedlings (DAS)

| Codes | Treatments | Ability to sprout (%) |
|---|---|---|
| A | Control | 96 |
| B | Chitosan nano 50 ppm | 90 |
| C | Chitosan nano 100 ppm | 96 |
| D | Chitosan nano 200 ppm | 94 |
| E | Silica nano 50 ppm | 93 |
| F | Silica nano 100 ppm | 98 |
| G | Silica nano 200 ppm | 100 |
| H | Chitosan nano 50 ppm+silica nano 50 ppm | 80 |
| I | Chitosan nano 100 ppm+silica nano 100 ppm | 100 |
| J | Fungicide | 93 |

of nano-silica concentrations and chitosan is effective as an alternative in seed coating methods. Seed coating could improve the appearance of seeds, increase shelf life, reduce the risk of contracting diseases from surrounding seeds and can be used as antioxidants, anti-microbials, repellents, antagonistic microbes and regulate growing substances[26].

**Growth of chilli sprouts:** Based on Table 6, the germination of chilli seeds percentage below 92.5% was the treatment of nano chitosan 50 ppm and nano chitosan 50 ppm+silica 50 ppm. The phenomenon that caused the treatment was infection with the fungus *Colletotrichum* sp., was decreased the viability of the seeds as a result. Observations of the high and long roots of chilli seed sprouts showed that formulations of nano-silica and chitosan single and mixed were able to increase both component growth in several treatments. Support from a previous study of *in-vitro* chitosan-silica nanocomposite tests showed the effect against the grey mould of table grapes up to 100%[27]. Because of the effectiveness of synergistic formulation between chitosan and silica nano, the combination can be used as fruit coating such as longan[28].

Table 7 showed that the average height of seed sprouts in each is lower than the control treatment except in the treatment of nano-silica 100 ppm and fungicides. Nano-silica treatment is 100 ppm higher compared to other treatments because nano-silica can support plant growth by increasing photosynthesis and resistance to biotic and abiotic changes.

Table 7: Growth component of the chilli on 12 DAS

| Codes | Treatments | Height (cm) | Root length (cm) | Number of leafy sprouts |
|---|---|---|---|---|
| A | Control | 4.1$^{e}$ | 2.8$^{e}$ | 2$^{ab}$ |
| B | Chitosan nano 50 ppm | 2.6$^{ab}$ | 1.3$^{a}$ | 1$^{a}$ |
| C | Chitosan nano 100 ppm | 3.0$^{abc}$ | 1.2$^{abc}$ | 2$^{ab}$ |
| D | Chitosan nano 200 ppm | 3.2$^{abcd}$ | 1.6$^{abcd}$ | 7$^{ab}$ |
| E | Silica nano 50 ppm | 3.5$^{bcde}$ | 2.4$^{bde}$ | 4$^{ab}$ |
| F | Silica nano 100 ppm | 4.0$^{de}$ | 3.2$^{be}$ | 19$^{c}$ |
| G | Silica nano 200 ppm | 3.4$^{cde}$ | 1.3$^{bde}$ | 5$^{ab}$ |
| H | Chitosan nano 50 ppm+silica nano 50 ppm | 3.8$^{abcd}$ | 1.2$^{abcd}$ | 8$^{b}$ |
| I | Chitosan nano 100 ppm+silica nano 100 ppm | 2.7$^{ab}$ | 3.6$^{ab}$ | 1$^{a}$ |
| J | Fungicide | 4.1$^{e}$ | 2.8$^{e}$ | 21$^{c}$ |

Same letter on one column in the table shows the data is not significantly different from the controls based on Duncan's 5% multiple distance test

## CONCLUSION

To conclude, both single and mixed nano chitosan and silica potentially suppress colony growth, conidia germination and suppression of anthracnose disease incidence by more than 90%. Red chilli anthracnose disease growth and inhibition decrease along with the increasing concentration of chitosan and silica nanoparticles. In addition, the lower germination was determined by the higher the number of concentrations. This research has a limitation on the proof of in-field testing, therefore, we suggest further testing is needed regarding the inhibitory power of the formulation on disease development of chilli plants in the field and also another test to understand the mechanism of action through gene expression and enzymatic activities.

## SIGNIFICANCE STATEMENT

This study discovers that chitosan and silica nanoparticles can be beneficial as an alternative control against anthracnose disease (*Colletotrichum* sp.). This study will help the researcher to uncover the critical areas of controlling anthracnose disease in the development and growth of the red chilli plant that many researchers were not able to explore. Thus a new theory on single and combination between chitosan and silica nanoparticles and possibly other combinations, may be arrived at.

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