Effect of Chitosan on Morphological Change Of *Colletotrichum capsici* (Sydow) Butler & Bisby

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INTRODUCTION

Background

Worldwide, postharvest losses have been estimated at 50% and much of this is due to fungal and bacterial infection. One of the important funguses that attack the postharvest product is fungus *Colletotrichum capsici* (Sydow) Butler & Bisby. This fungus caused anthracnose disease (Figure 1 and 2).

The symptom of anthracnose is black lesion, usually sunken caused by imperfect fungi that produce conidia in ecervuli. Conidia are borne on acervuli, witch are erumpent, cushion-like masses of conidiophores. The conidia are hyaline, one celled, avoid to oblong. The mycelium of pathogen is septate , inter-and intracellular. Acervuli and stroma in the stem are hemispherical and 70-120 µ in diameter. Setae are scattered and dark brown. The tips are light brown and several septate and up to 150 µ in length. Conidiophores are aseptate and unbranched. Conidia in mass appear light pinkish in color. Conidia are borne singly at the tips of the conidiophores. Individually they are hyaline, unicellular and caved with narrowed ends. These measure 7-28 x 3-4 µ. This is the characteristic oil globule in the center of each conidium. Conidia germinate in water within four hours. The germ tube soon forms an appressorium (Mehrorta, 1980).

Figure 1. The simptom of anthracnose disease on chili.

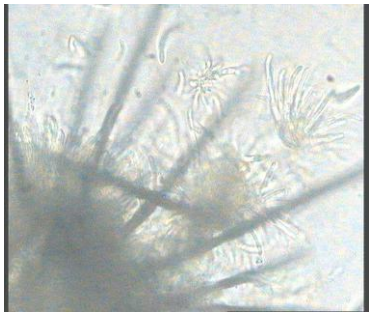

Figure 2. Structure of *C. capsici* from chili fruit.

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However, growing concerns over the presence of chemical residues in the food chain, the development of fungicide-resistant strain of postharvest pathogen, and the revocation of registration of some of the more effective fungicides, have generated an interest in the development of safer alternatives to synthetic fungicide that are both effective and economically feasible (El-Ghaouth, 1997).

Control of postharvest decay was also reported with chitosan. Chitosan, a cationic polysaccharide, is a natural material that appears to play a dual role by interfering directly with fungal growth and by activating several biological processes in plant tissues. Chitosan known as antifungal for several fungi, elicitor of many plant defense enzymes, inhibitor for proteinase, and synthesizes of lignin.

According to Benhamou (1992), chitosan was found to inhibit radial growth of *Fusarium oxysorum* f.sp. *radicis-lycopersici* with an optimal effect at concentrations ranging from 3 to 6 mg/ml. Light microscope observations showed that chitosan induced morphological changes, including hyphal swelling and distortion.

Objectives

The objectives of this research were to determine the direct effect of chitosan on hyphae radial growth, conidia germination period and interaction between chitosan concentration and hyphae radial growth of *C. capsici* (in-vitro assay).

MATERIALS AND METHODS

Chitosan preparation:
This research used the pure chitosan (prepared in VEDCA-Lab.). Sheets of chitosan were dissolved in 2.5% acetic acid and adjusting the pH to 5.6 with 2 N KOH Five percent (5%) solution of chitosan was solved in 5% acetic acid solution (Figure 3).

Fungal cultural and growth condition:

Pure inoculums of *C. capsici* was prepared through serial purification from chili fruit infected by *C. capsici* and mantained on Potato Dextrose Agar (PDA) and incubated in 37^0C incobator in dark place (Figure 4).

Figure 3. Resource of chitosan was from shrimp shell

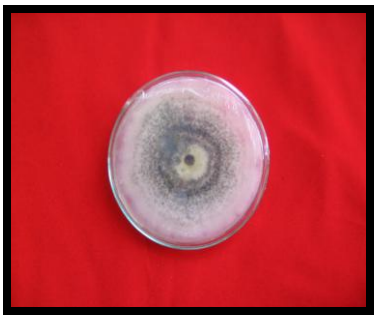

Figure 4. Pure inoculum of *C. capsici*

Effect of chitosan on colony diameter growth (antifungal assay)

Potato Dextrose Agar (PDA) was prepared with 0.025%, 0.3% , 0.75% and 1.75% chitosan concentration and control. Every PDA plates was seeded with 5-mm-diameter mycelia plug taken from 9-days-old *C. capsici* culture. The treatments were repeated three time. Inoculated plates were incubated at room temperature in the dark, and fungal growth was recorded at 1-day intervals until the control (PDA without chitosan) reached the edge of the plate. Growth inhibition is expressed as the percentage of inhibition of radial growth relative to the control.

Effect of chitosan on the morphological change of *C. capsici*

Colletotrichum capsici did not produce conidia in PDA Plate. Conidia were produced in Potato Dextrose Chili (PDC-5) liquid medium. Culture of 9-days-old *C. capsici* (5 mm) was seeded in 10 ml PDC. Inoculated PDC flask were cover with aluminum foil and incubated in shaker-water-bath 37^0C (9 days). Conidia suspension (50 µl from ± 10^6/ml) were deposited on the surface of microscope slides covered with a thin layer of PDA Chitosan 0.025, 0.30, 0.75, 1.75% and control (PDA). Inoculated slide were kept in a moisture chamber (sterile chamber with wet tissue layer bottom). Two days after inoculation, all slides were examined by light microscope to asses morphological changes of hyphae.

Effect of chitosan on conidia germination.

Conidia suspension (2 ml from ± 10^6/ml) was poured into 4 ml PDC-0.5 liquid medium (control) and PDC-0.5-Chitosan-0.75. Every treatment was dropped on specific object glass and the conidia germination was recorded by CCTV microscope.

RESULTS

Effect of chitosan on colony diameter growth (antifungal assay)

Chitosan significantly ($P<0.05$) reduced the colony diameter growth of.*C. capsici* at concentration 0.75-1.75% (Table 1, Figure 5). The relative growth inhibition 18.90 – 56.67%. The colony of pathogen at chitosan concentration 0-0.30% developed actively until by day 9 but at chitosan concentration 0.75-1.75% were halted. This research

continued to growth pathogen colony until 14 days, the colony diameter were not growth.

Table 1. Effect of Chitosan on Colony Diamater Growth and Relative Growth inhibition

No	Treatments	Colony Diameter (cm)	Relative Growth Inhibition (%)
1	Control	9.0a	0.00a
2	Chitosan 0.025%	8.4a	6.66a
3	Chitosan 0.30%	8.2a	8.90a
4	Chitosan 0.75%	7.3b	18.90b
5	Chitosan 1.75%	3.9c	56.67c

Means marked with the same letter do not differ significantly ($P<0.05$).

Figure 5 : Colony diameter of c. capsici on chitosan treatment in 9 days after inoculation. C-0 : control, C-1: chitosan 0.025%, C-2: chitosan 0.30%, C-3: chitosan 0.75%, and C-4: chitosan 1.75%.

The linier regression between chitosan concentration and *C. capsici* colony diameter was $Y= 8.93 -2.74 x$ with $R^2 = 96.3 \%$ ($P<0.05$). Every one percent of chitosan in PDA decreased 2.74 cm colony diameter (Figure 6).

Effect of chitosan on the morphological change of *C. capsici*

Microscopic observation show the a coagulation in the fungus cytoplasm characterized by the appearance of small vesicles in hyphae treated by chitosan 0.75 and 1.75% (Figure 7 a,b,c). At the control treatment (PDA) grew normaly but at all chitosan concentration, hyphae grew in abnormal shapes. Chitosan 0.75 and 1.75% induced increase in hyphal swelling, cell lysis and hyphal distortion.

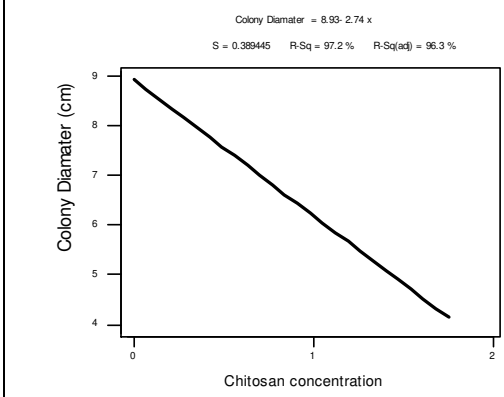

Figure 6. Linier regression between chitosan concentration and pathogen colony diameter (cm)

Figure 7. Morphological change of *C. capsici* at different chitosan concentration. (a) Normal hyphae on PDA had a compact cell wall (b) and (c) Distortion hyphae on PDA-Chitosan 0.75% and PDA-Chitosan 1.75% had swollen hyphae, lyses and disorganization hyphae.

Conidia germination period

In control treatment (PDC-0.5) conidia germinated at 9 hours after inoculation and developed germination tube but no appressorium. In the chitosan treatment (PDC-0.5-Chitosan-0.75%) conidia germinated at 68 hours after inoculation and germ tube develops an appressorium or directly developed an appressorium (Figure 8).

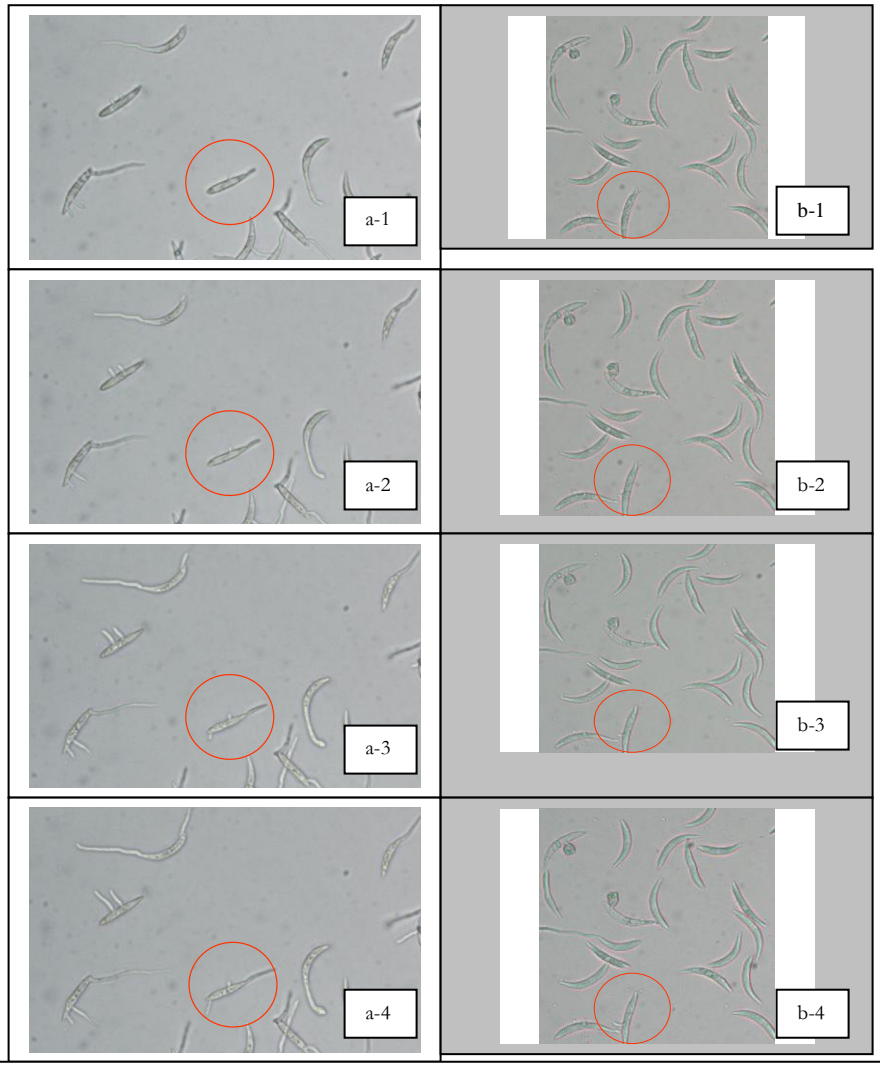

Figure 8. Germination process of *C. capsici*. (a) Conidia germination process in PDC-0,5 (kontrol) was 9 hours. (b) Conidia germination process in PDC-0,5-Chitosan 0,75% was 68 hours.

DISCUSSION

In commercial agriculture, chitosan remains relatively unexploited, even though its specific its specific properties such as bio-degradability, antimicrobial potential, and elicitor activity. In this research show that *C. capsici* suppression by chitosan. Chitosan was effective in inhibiting the growth of *C. capsici* in PDA-Chitosan. The inhibitory property of chitosan is well-known and has been demonstrated against several pathogenic fungi.

Light microscope investigations revealed that growth inhibition of *C. capsici* as a response to chitosan was accompanied by marked cellular change. Chitosan is effective in halting fungal growth.. The regression equation between chitosan concentration and dereasing of colony diameter of *C. capsici* is $Y = 8.93-2.74 x$, $R^2= 96.3\%$. Every one percent of chitosan concentration in PDA decreased colony diameter 2.74 cm. Chitosan inhibited growth of *C. capsici* colony diameter.

Chitosan is not only effective in inhibiting of fungal growth but also induces marked morphological change like hyphal swelling, lysis, distortion, structure alteration. Hyphal cells grown in the present of chitosan showed alteration in plasma membranes and modification of the cell walls (abnormal cell walls). How ever considering the polycationic properties of chitosan, chitosan induced alteration of the plasma membrane maybe largely responsible for the observed morphological and structural changes. Because the alternating orientation of positively charged glucosamine units along the polymer, chitosan may readily interfere with negatively charged residues of macromolecules exposed at the fungal cell surface. It is known that changes in lipid or phospholipids composition of the fungal plasma membrane permeability by altering the fluidity properties.

According to Benhamou (1992), Ultrastructural changes observed in chitosan-treated cells induced alteration of the plasma membrane and pronounced aggregation of the cytoplasm. Chitosan stimulated the activities both of ß-1.3-glucanase and chitosanase of *F. oxysporum* f. sp. *radicis-lycopersici*. The possible modes of action of chitosan are discussed relative to morphological and ultrastructure alteration, abnormal deposition of wall-like material, and the enhance enzymatic activities.

Chitosan was effective to postpone conidia germination. The speculation of this phenomenal was chitosan – induced alteration in the permeability of the conidia of *C. capsici* and have promoted internal osmotic imbalance. Chitosan is a positively polycationic, in other hand at the conidia cell walls surface is negatively charged. Chitosan was compatible with conidia surface and may enter the conidia and changed the metabolism. The exact mechanism involved in this process is still discussed. According to El-Ghaouth (1992), chitosan inhibited germinating process and colony growth of *Botrytis* and *Rhyzoctonia* on Strawbery.

CONCLUSSION

In conclusion, this experiment demonstrate that colony diameter of *C. capsici* was inhibited by chitosan 0.75-1.75%, more chitosan concentration would inhibit fungal growth. Chitosan changed the morphological of hyphae of *C. capsici* including lysis of hyphae, swelling and cell disorganization. Chitosan inhibited germ tube development of *C. capsici.*

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