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# The Test of Chitosan Concentration Against *Fusarium oxysporum* Fungus that Causes Fusarium Basal Rot Disease *of* Shallot by *In Vitro*

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***Abstract***

*The use of chemical fungicides continuously and not according to the rules can endanger the environment. Therefore, it is necessary to carry out alternative control using chitosan organic chemical fungicides. This study aims to test and obtain the concentration of chitosan that can inhibit the growth of F. oxysporum that causes FBRt disease in shallots in vitro. The treatments tested were: several concentrations of chitosan 0 g.l$^{-1}$ , 5 g.l$^{-1}$ , 10 g.l$^{-1}$ , 15 g.l$^{-1}$ , 20 g.l$^{-1}$ , 25 g.l$^{-1}$ , 30 g.l$^{-1}$ . The data obtained were analyzed statistically with ANOVA using the SPSS program. further tested with Duncan's New Multiple Range Test (DNMRT) 5% level. The results showed that the application of chitosan concentration of 5 g.l$^{-1}$ , 10 g.l$^{-1}$ , 15 g.l$^{-1}$ , 20 g.l$^{-1}$ , 25 g.l$^{-1}$ , 30 g.l$^{-1}$ was able to inhibit the growth of F. oxysporum Chitosan concentration of 20 g.l$^{-1}$ has a more capable ability in inhibiting the growth of fungi F. oxysporum causing F. oxysporum disease. oxysporum causing FBR disease on shallots in vitro with an average diameter of 76.56 mm, 14.92% inhibition. and able to inhibit the length and width of macroconidia of F. oxysporum fungus.*

*Keywords: Shallot, Fusarium Basal Rot, Chitosan*

## 1. Introduction

Indonesia, including Riau, is an archipelago whose seas and rivers are larger than the land, so Riau produces shrimp, crabs and the like. As the demand for frozen shrimp for export increases, the problem of shrimp shell and head waste is quite large. Pollution of the environment is caused by this garbage. The debris that results from this underutilization of shrimp waste pollutes the ecosystem.

Enhancing the quality of shrimp waste processing into a product with high economic value and wide applications is required to provide shrimp trash added value. For comparison, shrimp waste may be converted into chitin in nations like America, Japan, and Switzerland, in shrimp shell waste contains chitin by 20-50%. This chitin is the most natural polymer in the world after cellulose (1). Chitin can then be processed into kotosan (2) **,** chitosan is further processed into chitosan nanoparticles, chitosan and chitosan nanoparticles. Chitosan nanoparticles are anti-microbial, including anti-fungal (3), so they can be used as organic funsida and can be used in shallot cultivation (*Allium ascalonicum* L.).

Shallots are one of the horticultural crop commodities that have many benefits for the community. Shallots can be utilized as raw materials for the food processing industry, cosmetics and medicines. Shallots also have benefits in the health sector, one of which has the ability to prevent the growth of cancer cells (4). The many benefits of shallots cause shallots to be cultivated by many farmers, but shallot production in Indonesia, especially in Riau, is still relatively low.

The (5) reported that shallot production in Riau in 2021 was 329 tons with a planting area of 67 ha and productivity of 4.91 tons/ha. This figure is relatively low when compared to other provinces such as Aceh with a productivity of 8.52 tons/ha, North Sumatra 12.44 tons/ha, West Sumatra 14.44 tons/ha. A number of causes contribute to the low output of shallots, particularly in the Riau area: poor growing practices, the use of subpar seeds, difficult environmental conditions, insect assaults, and disease problems.

*Fusarium Basal Rot* FBR disease is an important disease of shallot plants (6)**.** This disease was found with a high attack rate in shallot development areas in Sungai Geringgingt**,** Kampar Regency (7). FBR is one of the diseases that is often found in shallot plantations in Riau and is an important disease in shallot plants (8) . The intensity of *F. oxysporum* attack on shallot plants reached 80.51% in Bungaraya Village, Siak Regency, Riau Province. Therefore, control must be carried out so that the spread of the disease does not continue to expand (9).

The symptoms of FBR disease-affected onion plants include yellowish green leaves that twist from the leaf blade tip to the base of the leaf.

Plants appear to wilt due to inhibition of growth, followed by changing the color of the leaves to brown, dry and finally the plant dies. **The** bulbs of dwarfed shallot plants are brownish and watery (10)**.**

The continuous and inappropriate use of synthetic chemical fungicides can have a negative impact on the environment, including the death of biological agents, the accumulation of residues in plants, increased health risks for users, and the potential for pathogen resistance (11) . Therefore, an alternative control is needed by using chitosan organic fungicides.

A polysaccharide formed from chitin is called chitosan.a substance found in the shells of crustaceans. Chitosan has certain properties that make it useful in various fields, including in agriculture, as an organic fungicide. Chitosan is the result of deacetylation of chitin (12). According to (13), chitosan at a concentration of 1.1% can inhibit colony diameter, conidia number, and 100% of the Conidia germination of Colletotrichum capsici. According to Rosmaladewi et al. (2020), Rhizoctonia solani Khun's mycelial growth and sclerotia development might be inhibited by applying chitosan at a concentration of 1.5% (15 g.l-

## 2. Research Methods

### 2.1 Trial Design

The research was conducted observationally and experimentally. Observational research is on macroscopic and microscopic characterization of *F. oxysporum* before and after chitosan application. Experimental research is on the inhibition test of several concentrations of chitosan against *F. oxysporum* isolated from shallots and the length and width of *F. oxysporum* macroconidia after chitosan application. A fully randomized design (CRD) with seven treatments and four replicates was used to perform the experimental investigation, yielding 28 experimental units, each experimental unit consisting of two Petri dishes. The treatments used in this study were chitosan K0: 0 g.l-1, K1: 5 g.l-1, K2: 10 g.l-1, K3: 15 g.l-1, K4: 20 g.l-1, K5: 25 g.l-1, and K6: 30 g.l-1 are the concentration tests that make up this set.

Data obtained from isolation and identification were analyzed descriptively and presented in tables and figures. Data on the diameter of F. *oxysporum* colonies isolated from shallot seeds on PDA medium, the percentage of chitosan inhibition of *F. oxysporum* fungal growth on PDA medium before and after application of various concentrations of chitosan and the length and width of macroconidia after chitosan application were analyzed by variance analysis and to compare the mean values between treatmentsFurther tests were run using the SPSS program and the Duncan's New Multiple Range Test (DNMRT) at the 5% level.

### 2.2 Research Implementation

#### 2.2.1 Rejuvenation of *F. oxysporum*

*F. oxysporum* isolated from shallot roots was re-grown on a petri dish containing PDA media by taking the fungal mycelium using a sterile ose needle. The *F. oxysporum* culture was incubated in an incubator for ± 7 days, then rejuvenated until homogeneous isolate growth was obtained. The rejuvenation of F. *oxysporum isoforms* was carried out in the LAFC.

#### 2.2.2 Preparation of chitosan

The chitosan obtained and used is that which has a deacetylation degree of 95%. The chitosan used was homogenized with 1% acetic acid as much as 10 ml weighed using digital scales as much as 0, 05 grams for treatment K1, 0.1 grams for treatment K2, 0.15 grams for treatment K3, 0.2 grams for treatment K4, 0.25 grams for treatment K5, 0.3 grams for treatment K6. The chitosan was then put into *aluminum foil* and labeled.

#### 2.2.3 Preparation of chitosan solution

The chitosan that has been weighed respectively was put into a test tube containing 10 ml of 1% acetic acid and then homogenized using a *vortex mixer*. Preparation of chitosan solution for treatment was pouring the chitosan solution using a micropipette according to the concentration of each treatment as much as 400 µl into 10 ml of liquid PDA media (temperature ± 40˚c) in a Petri dish. Petri dish was shaken so that the chitosan can be mixed well with PDA.

#### 2.2.4 Inhibition test of several concentrations of chitosan against *F. oxysporum in vitro* and measurement of macroconidia length and width

Inhibition test of several concentrations of chitosan against *F. oxysporum* was conducted *in vitro.* Fungal inoculation was carried out in the LAFC by growing fungal isolates that had been obtained from shallot root isolation. Isolates were taken using a 5 mm diameter *cork borer* and placed in the center of the PDA medium that had been given each treatment. The growth of fungal colonies was observed every day until the colonies on the petri dish without chitosan treatment ($K_0$ ) had filled the petri dish.

After completing the observation of the inhibition test, the *F. oxysporum* fungus that has been grown on several concentrations of chitosan is taken to measure the length and width of macroconidia. Macroconidia measurement uses the wet preparation method. Mycelia were seen using a 10x40 binocular microscope after being placed on a sterile glass item that had been dripped with

distilled water and covered with a cover glass. The NIS-Elements program was used for measurements on macroconidia.

**2,3 Observation**

**2.3.1 Morphological characteristics of causative fungi FBR**

Morphological characteristics include macroscopic and microscopic characteristics of shallots. Macroscopic characteristics of fungal colonies were carried out every day until the fungal colonies filled the petri dish (7 days). Visual macro observations were obtained by examining the mycelium's color and growth direction

(upward or sideways) and the texture of the mycelium (rough or smooth). Microscopic characteristics were carried out using a binocular microscope with a magnification of 10x40. Microscopic observation of *F. oxysporum* refers to the book *The Fusarium Laboratory Manual* (14).

**2.3.2 Colony diameter of *F. oxysporum* fungus after chitosan application on PDA media (mm).**

Measurement of the diameter of fungal colonies was carried out when the *F. oxysporum* fungal colonies in untreated petri dishes filled the petri dishes. Measurements were made using millimeter paper. Calculations were made by making vertical and horizontal lines intersecting exactly at the midpoint of the colony in the Petri dish. Petri dish at the bottom is given a line to facilitate the calculation of colony diameter. The method of calculating the diameter of fungal colonies is as follows:

$$D = \frac{d1 + d2}{2}$$

Description:
D = colony diameter of F. *oxysporum*
$d_1$ = vertical diameter of *F. oxysporum* colonies
$d_2$ = horizontal diameter of *F. oxysporum* colonies

**2.3.3 Percentage inhibition of chitosan against *Fungal* growth of *F. oxysporum* on PDA media (%)**

The percentage of inhibition of chitosan solution against the growth of *F. oxysporum* was calculated based on the measurement of the diameter of fungal colonies. The percentage of inhibition was calculated using the formula used, namely:

$$Daya\ Hambat\ (\%) = \frac{d - di}{d}\ 100\%$$

**2.3.4 Percentage inhibition of chitosan against *Fungal* growth of *F. oxysporum* on PDA media (%)**

The percentage of inhibition of chitosan solution against the growth of *F. oxysporum* was calculated based on the measurement of the diameter of the fungal colonies. The percentage of inhibition was calculated using the formula used, namely:

$$Daya\ Hambat\ (\%) = \frac{d - di}{d}\ 100\%$$

di = diameter of *F. oxysporum* colonies growing on PDA medium with chitosan concentration treatment
d = diameter of *F. oxysporum* colonies growing on PDA medium without chitosan concentration treatment

**2.3.5 Macroscopic and microscopic characteristics *F. oxysporum* after chitosan application**

Observation of macroscopic characteristics of *F. oxysporum* after chitosan application was carried out after the colonies on untreated petri dishes had filled the petri dishes. Observations were performed by looking at the mycelium's color and development direction (sideways or upward) and the texture of the mycelium (rough or smooth). Observation of microscopic characteristics of *F. oxysporum* after chitosan application was done by observing the shape of macroconidia and microconidia.

**2.3.6 Length and width of macroconidia *F. oxysporum* after chitosan application**

Observation of the size of *F. oxysporum* macroconidia after chitosan application was carried out on day 14 using the wet preparation method. Mycelia were placed on a sterile glass object that had been dripped with distilled water first, then covered with a cover glass and observed using a binocular microscope. Measurements of the length and width of macroconidia were made using the NIS-Elements application on a computer connected directly to the microscope.

**2.4 Data analysis**

Following the application of chitosan, data on the macroscopic and microscopic properties of Pestalotiopsis sp. were descriptively examined and displayed in tables and figures. Data on the diameter of *Pestalotiopsis* sp. fungal colonies on PDA media after chitosan application, chitosan inhibition of *Pestalotiopsis* sp. fungal growth on PDA media, and after applying chitosan, the length and breadth of Pestalotiopsis sp. conidia were statistically examined using variance analysis. Additional tests included the use of Duncan's New Multiple Range Test (DNMRT) at the 5% level.

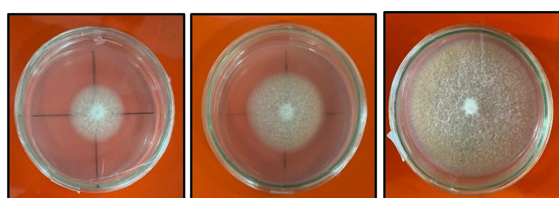
## 3. Results and Discussions

### 3.1 Morphological characteristics of the fungus F. oxysporum before chitosan application

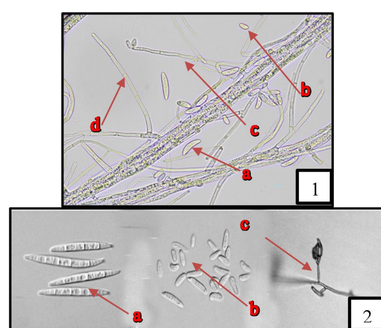
Macroscopic and microscopic characteristics of the FBR disease-causing fungi and F. oxysporum were obtained by observing the shape and size of the fungal structure. The observation results can be seen in Table 1, Figure 1 and Figure 2.

Table 1. Characteristics of the fungus F. oxysporum causing Fusarium basal rot disease on PDA media 14 hsi.

| Karakteristik Morfologi | Hasil Penelitian | Leslie and Summerell (2006) |
|---|---|---|
| **Makroskopis:** | | |
| Warna koloni | Putih/ putih krem | Putih hingga ungu pucat |
| Arah penyebaran | Tumbuh, Kesamping | Tumbuh, Kesamping |
| Bentuk miselium | Seperti kapas, miselium tumbuh jarang melimpah | Seperti kapas, bergumpal-gumpal, jarang/melimpah |
| **Mikroskopis:** | | |
| Bentuk makrokonidia | Berbentuk bulan sabit hampir lurus memiliki 3 sekat | Pendek-sedang, berbentuk bulan sabit sampai hampir lurus, memiliki 3 septa/lebih |
| Bentuk mikrokonidia | Elips | Berbentuk oval, elips, reniform (berbentuk seperti ginjal) tidak memiliki sekat |
| Bentuk hifa | Hifa bersekat | Hifa bersekat |
| Ukuran makrokonidia | 9, 55-21,8 x 1,8-4,72 µm | |
| Ukuran mikrokonidia | 3,2-7,81x 1,14 -3,8 µm | |



Macroscopic characteristics of F. oxysporum colonies causing Fusarium basal rot disease (A) 3 hsi, (B) 5 hsi, (C) 7 hsi




Microscopic observation of F. oxysporum isolation results before treatment (1) observation of F. oxysporum fungus, (2) microscopic F. oxysporum fungus according to Leslie and Summerell (2006). (a) macroconidia, (b) microconidia, (c) conidiophores, (d) filamentous hyphae

Observations of *F. oxysporum* fungi macroscopically and microscopically were made on days 7 and 14 after incubation or after the mycelium filled the Petri dish by looking at the growth of its colonies on PDA media. The results of macroscopic observations of *F. oxysporum* fungal colonies obtained were round, white in color accompanied by cream color (creamy white). Initially, the mycelium was white (Figure 1A). As the age increases, the color changes to beige (Figure 1B). These results are supported by the opinion of (14) , which can be seen in Table 1. According to (15) , The age of the culture affects how F. oxysporum colonies change color. Newer cultures are white in color. Each Fusarium species' characteristic color will emerge as the culture reaches maturity and then shift to purple, white, gray, or occasionally light brown. This is also in accordance with the research of (16) which showed creamy white colonies of *F. oxysporum* f. sp. *cepae*. The observation of the direction of mycelium growth is growing sideways, and the mycelium is smooth like cotton (Figure 1). This is in line with the research of (17)that *F. oxysporum* has a smooth colony texture like cotton and there are aerial mycelia. (16) also reported that the fungus *F. oxysporum* produces colonies that expand on the surface of the media.

Microscopic observations showed that *F. oxysporum* fungus has concentrated hyphae and has conidia in the form of macroconidia and microconidia. The macroconidia of *F. oxysporum* are almost straight crescent-shaped with tapered ends, having 3 partitions measuring 9.55-21.8 x 1.8-4.72 µm (Figure 2). Microconidia are elliptical, not insulated and have a size of 3.2-7.81x 1.14-3.8 µm. This is in accordance with the research of (18) who also reported that the macroconidia of *F. oxysporum* f. sp. *cepae* found had a size of 15-20 µm x 2.5-3 µm and had 3 partitions. Microconidia of *F. oxysporum* f.sp. *cepae* are oval, elliptical or kidney-shaped with a size of 2.5-15 µm x 2-3 µm and are not insulated.

### 3.2 Colony diameter of *F. oxysporum* fungus on PDA media (mm)

Chitosan treatment, showed different effects on the diameter of *F. oxysporum* colonies, this can be seen in Table 2 and Figure 3.

Table 2. Diameter of F. oxysporum colonies on PDA media after administration of several concentrations of chitosan 7 his

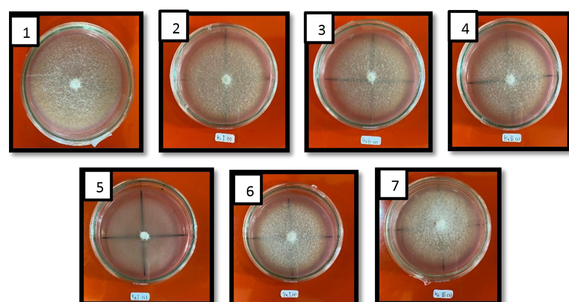
| Chitosan concentration | Diameter of *F. oxysporum* colony (mm) |
|---|---|
| 0 $g.l^{-1}$ | 90,00 a |
| 30 $g.l^{-1}$ | 83,50 b |
| 5 $g.l^{-1}$ | 82.75 bc |
| 10 $g.l^{-1}$ | 81.06 cd |
| 25 $g.l^{-1}$ | 81.06 cd |
| 15 $g.l^{-1}$ | 80,06 d |
| 20 $g.l^{-1}$ | 76,56 e |

Numbers followed by unequal lowercase letters are significantly different according to the DNMRT test at the 5% level.

The treatment with chitosan concentration of 20 $g.l^{-1}$ has the smallest mean colony diameter of 76.56 mm. The smaller mean diameter is the provision of chitosan concentration 15 $g.l^{-1}$ of 80.06 mm, not significantly different from the treatment of 25 $g.l^{-1}$

and 10 $g.l^{-1}$ which amounted to 81.06 mm. Giving chitosan concentration of 5 $g.l^{-1}$ has an average diameter of 82.75 mm, which is not significantly different from giving chitosan concentration of 30 $g.l^{-1}$ which is 83.50 mm. The treatment with chitosan concentration of 0 $g.l^{-1}$ has the largest average colony diameter of 90.00 mm and is significantly different from the diameter of *F. oxysporum* fungal colonies given other concentrations. This is because in the treatment without chitosan administration there is no chitosan which is anti-fungal so that the growth of *F. oxysporum* is not inhibited.

The use of chitosan concentrations of 0 $g.l^{-1}$ , 5 $g.l^{-1}$ , 10 $g.l^{-1}$ , 15 $g.l^{-1}$ , 20 $g.l^{-1}$ , 25 $g.l^{-1}$ and 30 $g.l^{-1}$ was able to inhibit fungal growth by reducing the average diameter of *F. oxysporum* colonies. The application of chitosan concentration of 20 $g.l^{-1}$ had the smallest average diameter compared to other chitosan test treatments. The effect of chitosan concentration on the growth of *F. oxysporum* can be seen in Figure 3.




Diameter of F. oxysporum colonies on PDA media 7 hsi by applying several concentrations of chitosan, 1 (K0) 0 g.l-1 , 2: (k1) 5 g.l-1 , 3: (k2) 10 g.l-1 , 4: (k3) 15 g.l-1 , 5: (k4) 20 g.l-1 , 6: (k5) 25 g.l-1 , 7: (k6) 30 g.l-1 .

The increase in chitosan concentration in the 25 g.l-treatment and the 30 g.l-1 treatment had a larger mean diameter compared to the 20 g.l-1 treatment (Figures 5 and 6). It is suspected that at concentrations of 25 g.l-1 and 30 g.l-1 the amine groups (NH2 -) that diffused into the fungal cells were excessive so that the inhibition of F. oxysporum was lower. This assertion is consistent with the findings of (19) that the administration of chitosan at excessive concentrations causes chitosan particles to diffuse excessively into fungal cells, causing inhibition of the growth of the fungus Alternaria solani Sor. lower. According to (20), the mechanism of chitosan inhibition is through particle diffusion into fungal cells followed by inhibition of DNA and RNA synthesis, and ultimately followed by fungal cell death.

### 3.2 Percentage inhibition of chitosan against F. oxysporum growth on PDA media (%)

Chitosan treatment, showed different effects on the percentage of chitosan inhibition of F. oxysporum growth on PDA media, this can be seen in Table 2 and Figure 3.

Table 3. Percentage inhibition of F. oxysporum colony growth after application of several concentrations of chitosan on PDA media 7 (hsi)

| Chitosan concentration | Mean percentage inhibition F. oxysporum colonies (%) |
|---|---|
| 20 g.l-1 | 14,92 a |
| 15 g.l-1 | 11,49 b |
| 25 g.l-1 | 9.92 bc |
| 10 g.l-1 | 9.79 bc |
| 5 g.l-1 | 8.05 cd |
| 30 g.l-1 | 7,14 d |
| 0 g.l-1 | 0,00 e |

Numbers followed by unequal lowercase letters are significantly different according to the DNMRT test at the 5% level.

The treatment of 20 g.l-1 produced the highest percentage of growth inhibition of F. oxysporum fungal colonies, namely 14.92%, which did not differ significantly from any of the treatments. Table 3 shows that the inhibition percentage of 15 g.l.-1 was 11.49 percent, which was not significantly different from the inhibition percentages of 25 g.l.-1 and 10 g.l.-1, which were 9.92 percent and 9.79 percent, respectively. With a chitosan concentration of 5 g.l.-1, the lower inhibition percentage of 8.05 percent was not significantly different from the concentration of 30 g.l.-1. which was 7.14%. The percentage of inhibition of F. oxysporum fungal colony growth in the treatment without chitosan (0 g.l-1 ) was significantly different and the lowest compared to other concentrations. This is due to the absence of anti-fungal compounds in the treatment so that the growth of F. oxysporum cannot be inhibited.

The percentage of inhibition of F. oxysporum fungi after the administration of chitosan concentration of 20 g.l-1 is better in inhibiting the growth of F. oxysporum fungal colonies. The administration of chitosan concentration of 20 g.l-1 is thought to cause the amine group (NH2 -) to be released from the long chain of chitosan and can already inhibit the growth of F. oxysporum fungi. This is in accordance with the statement of (21) who reported that the application of chitosan at different concentrations can inhibit the vegetative growth of pathogenic fungi. The application of chitosan with a concentration of 20 g.l-1 gave the greatest inhibitory effect on the second day after application. The application of chitosan can inhibit the growth of F. oxysporum fungal colonies because it contains anti-fungal compounds that activate the chitinase enzyme which can cause mycelium growth to be inhibited. In addition, chitosan can also cause disruption of fungal cell metabolism and destroy cell membrane structures. This is consistent with the findings of the study by (22).

Also reported that chitosan contains anti-fungus which can inhibit the growth of the cause of potato

late blight. These anti-fungal compounds can inhibit mycelial growth, cause metabolic disorders of Phytophthora infestans, destroy cell structures and membranes resulting in cell death.

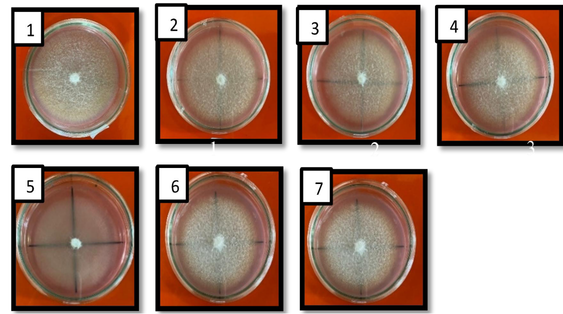
The higher concentration of chitosan applied did not result in higher inhibition in this study. Based on the observation, the concentration of 25 g.l-1 and 30 g.l-1 gave lower inhibition compared to the concentration of 20 g.l-1 . This is thought to be because the use of chitosan in very high amounts causes the amine groups contained in chitosan to be unable to bind to the fungal cell wall. This is in line with the research of (23), the use of chitosan with a chitosan concentration of 0.015% resulted in a percentage inhibition of 77.73% while the use of chitosan with a higher concentration of 0.025% resulted in a lower percentage inhibition of 23.61%. The percentage of inhibition produced in this study was smaller at 14.92% compared to the research of (21) using the same chitosan concentration of 20 g.l-1 can inhibit the growth of Colletotrichum musae fungal colonies by 81.20%. . This is in accordance with the opinion of.

### 3.4 Macroscopic and microscopic characteristics F. oxysporum after chitosan application

Macroscopic and microscopic characteristics of F. oxysporum after chitosan application by observing the shape and size of the fungal structure, can be seen in Table 4, Figure 4 and Figure 5.

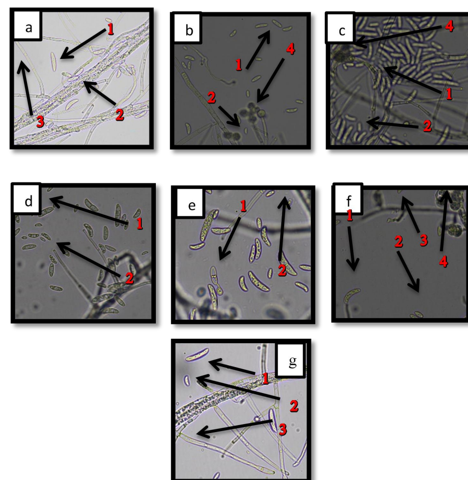
Table 4. Macroscopic characteristics of 7 hsi and microscopic characteristics of 14 hsi F. oxysporum after chitosan application

| Perlakuan | Pengamatan makroskopis dan mikroskopis | | | | | |
|---|---|---|---|---|---|---|
| | Warna Koloni | Arah Penyebaran | Bentuk miselium | Bentuk Makro-Konidia | Bentuk Mikro-Konidia | Klami-dospora |
| 0 g.l$^{-1}$ | Putih Krem | Tumbuh Kesamping | Seperti kapas, melimpah | Berbentuk bulan sabit | Elips | Tidak ada |
| 5 g.l$^{-1}$ | Putih Krem | Tumbuh Kesamping | Seperti kapas, tipis | Berbentuk bulan sabit | Elips | Ada |
| 10 g.l$^{-1}$ | Putih Krem | Tumbuh Kesamping | Seperti kapas, tipis | Berbentuk bulan sabit | Elips | Ada |
| 15 g.l$^{-1}$ | Putih Krem | Tumbuh Kesamping | Seperti kapas, tipis | Berbentuk bulan sabit | Mikro-konidia mengerut | Ada |
| 20 g.l$^{-1}$ | Putih | Kesamping | Seperti kapas, sangat tipis | Makro-konidia mengerut | Mikro-konidia mengerut | Ada |
| 25 g.l$^{-1}$ | Putih krem pekat | Tumbuh Kesamping | Seperti kapas, tipis | Makro-konidia mengerut | Mikro-konidia mengerut | Ada |
| 30 g.l$^{-1}$ | Putih krem pekat | Tumbuh Kesamping | Seperti kapas, tipis | Makro-konidia mengerut | Mikro-konidia mengerut | Ada |



Macroscopic characteristics of F. oxysporum fungi after application of several chitosan concentrations 1: 0 g.l-1 , 2: 5 g.l-1 , 3: 10 g.l-1 , 4: 15 g.l-1 , 5: 20 g.l-1 , 6: 25 g.l-1 , 7: 30 g.l-1 .

Giving chitosan concentration can cause changes in F. oxysporum fungi macroscopically and microscopically. Table 4 shows that the treatment of 20 g.l-1 fungal growth direction of F. oxysporum is sideways and does not grow upwards and is very thin. The use of chitosan as much as 20 g.l-1 can inhibit hyphal growth so that hyphae cannot grow upwards in contrast to the treatment without chitosan administration, hyphae grow abundantly upwards and sideways. It is believed that hyphal development is prevented due to the anti-fungal chemicals included in chitosan.
(23) reported that the use of chitosan can cause fungal growth to be inhibited and result in slow developing infections. This is because the fungal cell wall is negatively charged and the positively charged chitosan can bind.



Microscopic characteristics of F. oxysporum after application of several concentrations of chitosan. a: 0 g.l-1 , b: 5 g.l-1 , c: 10 g.l-1 , d: 15 g.l-1 , e: 20 g.l-1 , f: 25 g.l-1 , g: 30 g.l-1 . Ket: 1. macroconidia, 2. microconidia, 3. conidiophores, 4. chlamydospores.

Anti-fungal compounds contained by chitosan can also cause changes in the color of fungal colonies. Based on Table 4, it can be seen that the administration of chitosan with a concentration of 0 g.l-1 shows a creamy white colony color. An increase in concentration of 20 g.l-1 changes the color of fungal colonies to white as can be seen in Figure 4(5). The addition of chitosan to 25 g.l-1 and

30 g.l-1 caused the color of the colonies to become intense creamy white. This is thought to be a decrease in the amount of chitin in the hyphal wall of the fungus F. oxysporum due to the administration of chitosan which affects the color of the colonies. (24) stated that chitosan contains the enzyme β-1, 3 glucanase which can cause a decrease in the amount of chitin in the fungal hyphal wall so as to reduce colony growth and affect colony color changes.

Giving a chitosan concentration of 15 g.l-1 is able to cause the microconidia of the fungus F. oxysporum to shrink can be seen in Figure 5 (d2). Giving higher concentrations, namely 20 g.l-1 , 25 g.l-1 and 30 g.l-1 causes macroconidia and microconidia to shrink can be seen in Figure 5 (e1). This is thought to be because the compound content in chitosan can inhibit the biosynthetic process so that protein formation is inhibited and causes protein to not be used as a component in the preparation of the body structure of the fungus F. oxysporum. (25) reported that chitosan contains components such as (-NH2 ) which are positively charged and have electron pairs that can inhibit the biosynthetic triglycerides, phospholoids, and enzyme activity, oxidative, peroxidative in fungi.

### 3.5 Length and width of F. oxysporum macroconidia after chitosan application

Chitosan treatment, showed different effects on the length and width of F. oxysporum macroconidia after chitosan application, this can be seen in Table 5, Table 6 and Figure 6.

Table 5. Length of F. oxysporum macroconidia after chitosan application14 hsi

| Chitosan concentration | Macroconidia Length F. oxysporum fungus (μm) |
|---|---|
| 0 g.l-1 | 18, 63 a |
| 10 g.l-1 | 16, 59 b |
| 5 g.l-1 | 16, 10 b |
| 15 g.l-1 | 14, 94 b |
| 30 g.l-1 | 12, 27 c |
| 25 g.l-1 | 11, 84 c |
| 20 g.l-1 | 11, 26 c |

Numbers followed by unequal lowercase letters are significantly different according to the DNMRT test at the 5% level.

Table 5 shows that the length of macroconidia in the treatment of 20 g.l-1 which is 11.26 µm is not significantly different from the treatment of 25 g.l-1 and 30 g.l-1 which are 11.84 µm and 12.27 µm. Giving chitosan concentration to 30 g.l-1 affects the length of macroconidia to 12.27 µm Giving chitosan concentration to 15 g.l-1 affects the length of macroconidia to 14.94 µm, which is not significantly different from the treatment of 5 g.l-1 and 10 g.l-1 , namely 16.10 Giving chitosan concentration to 30 g.l-1 affects the length of macroconidia to 12.27 µm and 16.59 Giving chitosan concentration to 30 g.l-1 affects the length of macroconidia to 12.27 µm. The length of macroconidia in the treatment without chitosan (0 g.l-1 ) is significantly different compared to other chitosan treatments. This is due to the absence of anti-fungal compounds in the treatment so as not to affect the length and width of macroconidia of F. oxysporum.

Table 6. Macroconidia width of F. oxysporum after chitosan application14 hsi

| Chitosan concentration | Macroconidia Width F. oxysporum fungus (μm) |
|---|---|
| 0 g.l-1 | 3, 86 a |
| 10 g.l-1 | 3, 77 a |
| 15 g.l-1 | 3, 31 ab |
| 5 g.l-1 | 3, 23 ab |
| 30 g.l-1 | 3, 02 bc |
| 25 g.l-1 | 2, 85 bc |
| 20 g.l-1 | 2, 36 c |

Numbers followed by unequal lowercase letters are significantly different according to the DNMRT test at the 5% level.

Based on Table 5 and Table 6, it is known that the application of several concentrations of chitosan affects the length and width of macroconidia of F. oxysporum. Chitosan concentration of 20 g.l-1 gave better inhibition in inhibiting the length and width of conidia compared to the administration of other chitosan concentrations (Figure 6). This is thought to be because chitosan contains anti-fungal compounds that can inhibit fungal growth both vegetatively and generatively. (21) reported that the administration of chitosan at different concentrations can inhibit the vegetative and generative growth of the fungus Colletotrichum musae. the length and breadth of conidia, which are 10.71% and 19, 14%, respectively, were inhibited by chitosan at a concentration of 20 g.l-1.

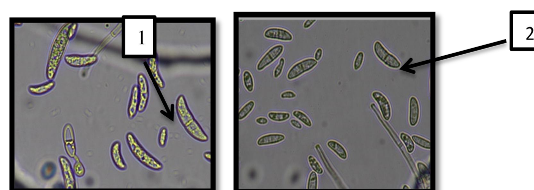



Image 6. Observation of macroconidia and microconidia size of F. oxysporum after application of 20 g/l chitosan, (1) shrinkage of macroconidia size, (2) shrinkage of microconidia size.

Inhibition of conidia length and width is also caused by chitosan which affects cell division so that it has a direct impact on the formation of fungal conidia, can be seen in Figure 5. The results of this study are in line with research (26) which reported that chitosan affects generative growth in the formation of conidia of the fungus Colletotrichum musae. The use of chitosan concentration of 20 g.l-1 showed greater inhibition of the number of conidia, length and width of the conidia of the fungus Colletotrichum musae. (24) also stated that the inhibition of colony growth, reduced number of

conidia, and size of fungal conidia is due to chitosan containing compounds that can cause a decrease in the amount of chitin in the fungal hyphal wall so as to reduce colony growth.

Disease control can be done using chitosan because chitosan contains anti-fungal compounds. According to (27) chitosan has an extremely potent positively charged amine functional group (NH2) that attracts negatively charged amino acid molecules, which are the building blocks of bacterial proteins. The electrostatic interaction between the positive and negative charges results in a permeability pressure across the membrane. This imbalance in the osmotic pressure within the cell prevents bacteria from growing.

## 4. Conclusion

The provision of several concentrations of chitosan has an effect on the growth of *F. oxysporum*. Chitosan concentration of 20 $g.l^{-1}$ has a better ability to inhibit the growth of *F. oxysporum* fungus that causes Fusarium basal rot disease on shallots *in vitro* with an average diameter of 76.56 mm with a percentage inhibition of 14.92%. Chitosan concentration of 20 $g.l^{-1}$ was able to inhibit the length and width of macroconidia of *F. oxysporum.*

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