The efficacy of chitosan to control nematode *Aphelenchoides besseyi* Christie through seed treatment

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Abstract. One of the pathogen that infect rice is white tip nematode (*Aphelenchoides besseyi*). *A. besseyi* is a seed-borne pathogen that can cause yield losses 30% to 50%. To overcome this problem, it is necessary to have an effective, efficient, and environmentally friendly control method. The purpose of the research was to study effectiveness of chitosan in controlling white tip nematodes. The experimental design used in this research was completely randomized design. The chitosan concentrations used in this experiment were 0% (control), 0.25%, 0.5%, 0.75%, and 1%. Seeds of each treatment were soaked for 15 min. Each treatment was replicate 5 times, with 25 seeds being used for each replication. Observations including the most effective soaking period, seeds germination, nematodes population, and symptom of morphological damage caused by nematodes. Observations of seed viability were carried out using paper rolls test and directly counting population of nematodes using stereo microscope. The best treatment concentration was determined by % seeds germination and lowest nematodes population. The result showed that chitosan with concentration 0.25% was effective to control *A. besseyi* and not significantly different with hot water treatment which is an effective control method to control *A. besseyi* to date. The treatment was also did not adversely affect to seed germination.

1. Introduction

Rice is an important food crop that has become the staple food for more than half of world population. During the period 2013-2015, unhusked rice production in Indonesia fluctuated by 71,279,709; 70,846,465; and 75,390,841 tonnes [1]. Efforts to increase rice productivity are hampered by several factors, one of which is the problem with pest and diseases. The nematode *Aphelenchoides besseyi* that can cause white tip disease has been reported worldwide and could cause damage and loss to rice production [2, 3]. *A. besseyi* is seed borne in anhydrobiosis condition during storage in warehouses and considered ectoparasitic [4]. As for other problems with nematode disease, hot water treatment is considered effective control for *A. besseyi* according to Supramana (personal communication), but there are no facilities and tools available in Indonesia for large scale treatment.
Chitosan is an amorphic solid with yellowish white color. Generally, chitosan is made from deacetylated crustacean chitin (outer shell) waste which has 70% of its acetyl group removed. In agriculture, chitosan is valued for its antimicrobial and antifungal properties [3]. For example, chitosan can control Colletotrichum capsici that causes anthracnose disease on chilli and Pseudoperonospora cubensis which causes downy mildew disease on melons [5, 6]. Chitosan is not only effective in halting the growth of pathogen, but also in altering the morphology, structure, and disorganisation of fungal molecular cell. Based on those knowledge, this research aimed to investigate the effectiveness of chitosan against A. besseyi.

2. Methods
This research was conducted in Plant Nematology Laboratory of Plant Protection Department, Faculty of Agriculture, IPB University on September 2018 until May 2019.

2.1. Preparation of chitosan solution
Concentration of chitosan used in this experiment were 1%, 0.75%, 0.5%, and 0.25%. Chitosan was obtained from 2 sources, commercially and from Department of Aquatic Products Technology (THP), Faculty of Fisheries and Marine Science, IPB University. Chitosan from THP was made by mixing 1 g of chitosan with 20 mL of acetic acid. After chitosan forms a lump, 1% distilled water was added to make 100 mL solution of 1% chitosan solution. This stock solution was used for serial dilution to make a concentration of 0.75%, 0.5%, and 0.25%. Preparation of chitosan solution from commercial product was conducted following the product instruction.

2.2. Seed treatment
Chitosan was applied by soaking rice seeds on chitosan solution for 15 min. A total of 10 chitosan treatments was evaluated, i.e. THP and commercial chitosan, each at 0% (control), 1%, 0.75%, 0.5%, and 0.25% concentrations. As treatment comparison, rice seed was soaked in warm water at 50 °C for 15 min. The seeds were planted on prepared media following the treatment.

2.3. Seed viability testing
Seeds viability testing was conducted using paper roll method. Three layers of paper were prepared and moistened with water then placed on plastic. Each seed that had been surface sterilized using 0.1% NaOCl, was rinsed 3 times with sterile water; then 25 seeds were placed on the paper in a zig zag pattern. Later on, the papers were rolled and placed on room temperature. Seeds viability observation was conducted after 7 d by measuring germination rate to observe the impact of treatments to seeds viability.

2.4. A. besseyi population observation
Treated seeds were extracted using modified Baermann method for 24 hr in dark condition, then extraction results were filtered using 400 mesh filter. Extracted nematodes were counted using hand counter and stereo microscope. Identification of A. besseyi was done morphologically using EPPO (2017) as reference. Population count was conducted using sampling method with each extraction replication counted 5 times. The population count used the following formula:

\[ N = \frac{V}{v} \times n \]

With, 
- \( N \) : total nematode population
- \( n \) : nematode species population counted on counting dish
- \( V \) : extracted nematode suspension volume (mL)
- \( v \) : Suspension volume on counting dish (mL)
2.5. Observation of A. besseyi body damage after chitosan treatment

Extracted A. besseyi nematode that has been treated were then observed under compound microscope connected to a computer. Observation made by fishing the nematodes beforehand using nematode fishing equipment and stereo microscope. Nematodes were then placed on object glass and covered with cover glass. Observations made by observing the damage that occurs in the body of A. besseyi.

2.6. Data analysis

This research was arranged in completely random experimental design consisting of 10 treatments with each treatment replicated 5 times. Data was tabulated in Microsoft Excel 2010 and analysed with analysis of variance (ANOVA) and continued with Tukey test at α=5% using IBM SPSS version 22 for Windows 10.

3. Result and discussion

Effect of seed immersion using THP chitosan showed insignificant differences to seeds viability compared to control treatment at all concentrations. Testing results of commercial chitosan showed only chitosan at 0.25% concentration that did not significantly differ to control treatment. Other concentrations have different viability values which were significantly different and lower than control. This might be due to the chemical composition of the commercial chitosan which reduce seeds viability on high concentrations.

Chitosan capability to increase germination rate is supported by gibberellin hormone content which is important for cell lysis and division plus the growth and elongation of plant stem during germination [7]. Based on previous research, chitosan contains IAA hormone, hence it has the ability to increase plant growth [8].

Experiment results using HWT method (Table 1) produce highest germination rate compared to other treatments. The high value differences was due to the usage of different seeds during replication. The new seeds were bought because the deteriorating condition of previous seeds due to infestation of Sitophilus sp., in addition to the seeds stock that was insufficient for the next stage of research.

HWT treatment produced the best result regarding seeds viability compared to control and other treatment (Table 1). The results are in line with previous research by which HWT treatment at 50 °C for 15 min did not affect seeds viability [9]. Based on the results of seeds viability test, seeds with THP chitosan treatment (1%, 0.75%, 0.5%, 0.25%), commercial chitosan treatment at 0.25%, and HWT treatment do not negatively impact seeds viability and the research will proceed to the next stage to find out the effectiveness in eliminating A. besseyi from rice seeds.

Extraction results (Table 2) showed that all seeds treatments given could lower the number of A. besseyi significantly compared to control, even though statistically chitosan THP 1%, THP 0.75%, and THP 0.5% treatments did not differ significantly. Chitosan solution with 1% concentration still has high concentration density, thus has difficulty in entering seeds [10]. Other than that, a too short immersion duration might also be a factor in determining the effectivity of chitosan in entering seeds and eliminating A. besseyi contained inside.

THP and commercial chitosan both at 0.25% concentration showed decent results and significantly differed with control treatment. The concentrations of those chitosan might be not too concentrated, thus have an easier time entering the seeds and infecting nematodes. The lower the concentration of chitosan, the higher the effectiveness in infecting A. besseyi inside the seeds. This is because when chitosan is dissolved it will form electron clouds, if the concentration is too high (in this research at 1%) then the electron clouds will roll up each other and obstruct the pathway to enter the seeds. If using a lower concentration of chitosan, then the electron clouds will elongate and strengthen each other, thus have an easier time in entering the seeds (Suptijah 2019 May 14, personal communication).

HWT produced the lowest number of A. besseyi compared to other treatments. This result was in line
with research done by [11] which stated that HWT at 50 °C for 15 min is effective in lowering the number of A. besseyi.

Table 1. Impact of immersion treatment to seed germination.

| Treatment | Germination ratea |
|-----------|------------------|
| Control   | 62.40b           |
| HWT       | 99.47a           |
| THP1      | 72.53b           |
| THP0.75   | 66.40b           |
| THP0.5    | 64.00b           |
| THP0.25   | 62.67b           |
| KOM1      | 25.60d           |
| KOM0.75   | 30.93d           |
| KOM0.5    | 44.80c           |
| KOM0.25   | 65.07b           |

a The numbers in the same column are followed by the same letters, showing no significant difference based on Tukey's further tests at α = 5%.
b HWT= hot water treatment, THP=chitosan obtained from THP, KOM= commercial chitosan, 0.25-1= % treatment concentration.

Microscopic observation results (Fig. 1) showed damages occurring on the body of A. besseyi which were caused by THP chitosan treatment at 0.25% and 0.5%, commercial chitosan 0.25%, and HWT. The internal organs could not be observed clearly due to the damages. Microscopic observation showed that A. besseyi was still alive during observation as indicated by its body movements. However the damages are expected to affect virulence and fecundity due to the condition of its internal organs. So, besides being nematicidal, chitosan can also be nematostatic.

![Figure 1. Symptoms of damage to the body of A. besseyi at THP chitosan at 0.25% treatment (a), THP chitosan at 0.5% treatment (b), commercial chitosan at 0.25% treatment (c), and hot water treatment (d).](image)

The mechanism of damage on a body of A. besseyi is possibly due by chitosan composition which has 3000 monomers. Each monomer will form electron cloud when dissolved. Each monomer has amide group which is positively charged and hydroxyl ion which is negatively charged. Each group bind to nematode body consisting of protein or polysaccharide which generally is negatively charged.
and caused a pull that irritates, damages, and lysis the body the internal organs. In addition, chitosan can enter nematode through damaged bodies and damage (Suptijah 2019 May 14, personal communication). Damage symptom on A. besseyi body due to HWT (Fig. 1d) was similar to the damage caused by chitosan. The body and internal organs were visually damaged due to lysis because of denaturation on the given temperature range.

Table 2. Number of extracted A. besseyi after seed treatment.

| Treatment | Nematode number (individual/400 seeds) |
|-----------|----------------------------------------|
| Kontrol   | 226.00a                                |
| HWT       | 62.00c                                 |
| THP1      | 132.00abc                              |
| THP0.75   | 172.67ab                               |
| THP0.5    | 171.33ab                               |
| THP0.25   | 116.00bc                               |
| KOM0.25   | 120.00bc                               |

*The numbers in the same column are followed by the same letters, showing no significant difference based on Tukey’s further tests at α = 5%.

b HWT= hot water treatment, THP= THP department chitosan, KOM= commercial chitosan, 0.25-1= % treatment concentration.

4. Conclusion

Based on this research, seeds immersion treatment using both THP chitosan and commercial chitosan at 0.25% concentration and hot water treatment at 50 °C for 15 min were effective in controlling A. besseyi. All 3 treatments showed insignificant statistical differences compared to those of other treatments and significantly different compared to those of control treatment. Even so, HWT still produced the best result compared to all other treatments by producing the lowest number of extracted A. besseyi which indicates the highest nematode elimination rate.

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