The Effectiveness of filtrate culture with *Fusarium spp.* fungi and against *Meloidogyne spp.* nematodes by *in-vitro* tomato plants

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Abstract. Parasitic nematodes are one of the essential plant pests that attack various types of cultivated plants. In Indonesia, 26 species of parasitic nematodes that attack food crops, horticulture and plantations have been identified. One such nematode, *Meloidogyne*, is the most destructive parasitic nematode. The utilization of other microorganisms as natural enemies of nematodes from the fungal group can be used as biological agents. We conducted this research at the Laboratory of Disease, Department of Pests and Plant Diseases, Faculty of Agriculture, Hasanuddin University Makassar. This study aims to determine the ability of *Fusarium spp.* Fungal isolate could be added with liquid media (Gliotoxin fermentation media (GFM), Potato dextrose broth (PDB), and water) and the time required to cause juvenile mortality II root-knot nematodes (*Meloidogyne sp.*). The results showed that the application of *Fusarium* isolate (*Fusarium spp.*) and the given liquid media was able to cause juvenile mortality of root-knot nematodes (*Meloidogyne sp.*). Moreover, the time required by *Fusarium spp.* Fungi and liquid media to cause nematode mortality was 24 hours, but the highest mortality occurred at 9 (jst) hours after application, namely 7.74 % at the BNJ test level of 0.05%.

1. Introduction
Parasitic nematodes are one of the essential plant-disturbing organisms that attack various types of cultivated plants. In Indonesia, 26 species of parasitic nematodes that attack food crops, horticulture and plantations have been identified. Among these nematodes is Meloidogyne. *Meloidogyne spp.* is one of the biggest causes of crop failure that occurs in Indonesia. Nematode infestation can cause significant yield losses. In general, the attack of nematodes causes damage to the roots because the nematodes absorb root cells so that the network vessels are disturbed. As a result, the translocation of water and nutrients is inhibited. Nematode attacks can also affect photosynthesis and transpiration [1]. During the last 50 years, control of nematodes using chemical nematicides (synthetic) has played a vital role as other control methods have not provided satisfactory results. So, it requires the development of natural or vegetable materials that are not harmful to the environment and humans who consume them.
2. Methods

2.1. Provision of Meloidogyne spp. larvae
The roots of tomato plants infected with Meloidogyne spp. were taken from the field, then washed and cut into 1-2 cm sizes, then crushed using Mortard and put into Erlenmeyer containing 0.5% NaOCl solution, then shake for 4 minutes. Then a solution is filtered using a nematode sieve (Mesh) sequentially 80, 100, 140, 200, and 500 mesh filters. The filtered solution is then rinsed using distilled water to remove the NaOCl solution.

The filters results are observed under a microscope to confirm the presence of eggs and larvae of Meloidogyne spp. After confirming that there is, the solution is put into Erlenmeyer and aerated for ten days. The counting of larvae is done by taking a sample of 1 ml and will be counted under a microscope repeated five times so that the average population per milliliter of the solution is obtained.

2.2. Provision of antagonistic fungi
Antagonistic fungal isolates were taken from healthy tomato plantations in the vicinity of plants that were indicated to be affected by the root-knot disease. The aim is to find out what kind of endophytic fungi are in the plant.

The plants obtained are then cleaned off the remaining dirt attached to the plant and washed, after which the roots of the tomato plants are cut into 1-2 cm sizes, then surface sterilization is carried out. After the root pieces are sterilized, they are transferred to a fungus culture medium, Potato Dextrose Agar (PDA), then five stored in a sterile room until the fungus on the root pieces grows.

2.3. Provision of filtrate culture
The fungi that grew in the culture media were taken as many as five explants using Cork Borer with a diameter of 8 mm, then put into Erlenmeyer, To, which contains Potato Dextrose Broth (PDB) liquid media and Gliotoxin Fermentation Media (GFM) liquid media. In order to manufacture PDB and GFM liquid media: 1) Control explant is inserted into Erlenmeyer, which contains distilled water as a solvent for endophytic fungi. 2) For PDB liquid media, the ingredients used are clean potatoes and then peeled and weighed as much as 200 grams, then boiled using 1,000 ml distilled water until the extract from the potatoes comes out, after that put them into Erlenmeyer then add 20 g of sugar. 3) For GFM liquid media, the materials used are 2gr of Ammonium tartrate, 2gr of KH2PO4, 1gr of MgSO4·7H2O, 0.01gr of FeSO4, and 25 grams of dextrose, all of which are put into Erlenmeyer and includes 1,000 ml of distilled water.

2.4. Identification of found fungus
We identified antagonistic fungi macroscopically and microscopically from all isolates from several sampling areas, including colony color, colony surface shape, and colony density. Microscopic observations included the shape of the hyphae, conidia, location of the conidia, and the hyphae’s particular structure.

2.5. In-vitro test of fungal filtrate culture against mortality of Meloidogyne spp.
Nematodes 20 ml of fungal culture extract was added to 100 ml Erlenmeyer, then 100 J2/10 ml larvae were added, so that in 30 ml there are 20 ml of fungal culture extract and in 10 ml there are 100 J2 larvae nematodes. Observations were carried out under a microscope for 2 hours, 6 hours, 12 hours, and 24
hours. To see whether the nematode is dead/inactive dead/inactive by calculating the mortality percentage of *Meloidogyne spp.* by using the following formula:

\[ M = \frac{a}{b} \times 100\% \]

- \(M\) = Mortality percentage (%)
- \(a\) = The total of dead nematodes
- \(b\) = Total of initial nematodes (before application)

### 3. Results and discussion

#### 3.1. Results

3.1.1. Identification of fungal isolates found. The obtained results from the isolation of fungi of tomato plants root soil and nematode eggs are two the fungal isolates that will be tested on the nematode larvae reared (figure 1).

![Figure 1](image1)

**Figure 1.** Fungus isolate. A) Red *Fusarium* (macroscopic), a) Red *Fusarium* (microscopic). B) White *Fusarium* (macroscopic), b) White *Fusarium* (microscopic).

3.1.2. In-vitro test of fungal filtrate culture against mortality of *Meloidogyne spp.* The results of antagonistic activity testing of each fungal isolate against larvae of the nematode *Meloidogyne spp.* Based on the observation time of 1 hour, 3 hours, 6 hours, 9 hours, and 24 hours, it is presented in table 1. The observation results of the antagonistic activity test of each fungal isolate in *Meloidogyne* larvae can be seen in figure 2.

![Figure 2](image2)

**Figure 2.** The effect of fungal filtrate on larvae of *Meloidogyne spp.* larva (a) normal and (b) damaged.
Table 1. The results of testing the filtrate culture with fungal isolates in the length of time observed.

| Time  | Treatment | Fungal isolate | FO1 (%) | FO2 (%) |
|-------|-----------|----------------|---------|---------|
|       |           |                | GFM     | PDB     |
| 1 hours| PDB       | 4.0            | 2.7     |         |
|       | AIR       | 3.3            | 4.0     |         |
|       | GFM       | 1.3            | 0.0     |         |
| 3 hours| PDB       | 18.0           | 14.0    |         |
|       | AIR       | 15.3           | 13.3    |         |
|       | GFM       | 14.4           | 4.7     |         |
| 6 hours| PDB       | 41.3           | 40.7    |         |
|       | AIR       | 39.3           | 38.0    |         |
|       | GFM       | 25.3           | 16.0    |         |
| 9 hours| PDB       | 76.7           | 76.7    |         |
|       | AIR       | 71.3           | 72.0    |         |
|       | GFM       | 49.3           | 36.0    |         |
| 24 hours| PDB      | 100            | 100     |         |
|       | AIR       | 59.3           | 66.0    |         |

Note: The numbers do not differ significantly in the BNJ continued test for the confidence level of 0.05.

3.2. Discussion

The fungi found showed an antagonistic activity. Fungi are parasites of *Meloidogyne spp*. Namely *Fusarium* sp. Apart from being a parasite, the larvae of *Fusarium sp.* also act as producers of compounds with nematicide activity. The test results showed that the *Fusarium sp.* fungus also produces nematicides, which can be seen in *Meloidogyne sp.* larvae (Table 1). Previous study conducted [7] stated that the filtrate of *Fusarium sp.* can damage eggs, inhibit hatching and kill the infective larvae of *Meloidogyne spp.* since the *Fusarium sp.* fungus produces compounds in the form of enzymes or toxins that have activity as nematicides.

According to [8], *Fusarium, Verticillium, Aspergillus, Penicillium,* and *Paecilomyces* fungi, are easily found in soil with a high content of organic matter and are effective in controlling nematodes. The control mechanism is thought to be due to the influence of the toxin produced by the fungus, which harms the life of parasitic nematodes. Other compounds produced by fungi that can kill nematodes include antibiotics. Some antibiotic-producing fungi include *Penicillium* (penicillin, griseofulvin), *Chepalosporium* (cephalosporin), and several other fungi such as *Aspergillus* (fumigasin), *Chaetomium* (chetomin), *Fusarium* (javanisin), *Trichoderma* (gliotoxin), and others [9].

*Fusarium sp.* fungus is very active in using hyphae to infect the nematode's body [10] so that larvae and adults die with the contents of the body depleted (Figure 2). [11] stated that the *Fusarium oxysporum* fungus and *Paecilomyces lilacinus* can be isolated from the eggs of *Meloidogyne sp.* and after being tested in vitro, it could infect *Meloidogyne sp.* [9] found *Fusarium oxysporum* isolated from *H. schachtii* eggs and sugar beet soil in California, and after being tested, it shows that these two fungi could attack the eggs of *Meloidogyne sp.*

4. Conclusions

It can be concluded that: The contents of *Fusarium sp.* can produce antibiotic compounds that act as nematicides. The applied filtrate culture treatment can increase the applied fungi's growth to produce nematicide compounds quickly.
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