Screening of biological control agent fungi against peanut stem rot (*Sclerotium rolfsii* Sacc.) in the peatlands of Kuala Pesisir Nagan Raya, Aceh, Indonesia

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**Abstract.** Peanut stem rot disease caused by *Sclerotium rolfsii* Sacc. is a problem that needs special attention in peatlands. Peatlands that are wet, acidic and contain high organic matter should be able to support and increase the pathogenicity of *S. rolfsii*. However, this is not the case for the peatlands of Kuala Pesisir Nagan Raya, which have been other components that prevent these pathogens from developing. Here we try to examine the biological elements, namely the existence of biological control agents from the fungal group, which generally like the same environmental conditions as pathogens. This study aims to obtain peat soil antagonistic fungi that can control *S. rolfsii*. This research was conducted in Kuala Pesisir, Nagan Raya Regency, Aceh, Indonesia. Research activities include isolation, morphological identification, pathogenicity testing, antagonist testing, and molecular identification. The results obtained 46 fungal colonies from three sources, namely weed rhizosphere around peanut farming, peanut rhizosphere, and groundnut roots. The results showed that there were antagonistic fungi that could suppress *S. rolfsii*, namely *Trichoderma asperellum*.

1. **Introduction**

Peanut stem rot disease caused by *Sclerotium rolfsii* Sacc. is one of the essential diseases in peanut (*Arachis hypogaea* L.). The condition causes widespread damage in many countries, including Indonesia, reducing crop yields by 10 – 25%. Pod yield losses can be over 80% in large amounts of *S. rolfsii* infested land [1]. The centre of peanut production in Indonesia, such as in East Java, disease severity due to *S. rolfsii* reaches around 8.61% [2], even in West Lombok the seriousness of the disease can get 80 - 90% [3].

The pathogenic fungus *S. rolfsii* can exist in soil without a host plant by utilising plant debris in the ground. If environmental conditions are no longer favourable, then this fungus can form sclerotia [4]. Peatlands are suitable for *S. rolfsii* activities due to the humid conditions of the peatlands [4], organic
matter from abundant plant debris [5], and the soil is acidic [6],[7], thus providing environmental conditions that support the development of these pathogens. However, this did not happen in the peatlands of Kuala Pesisir, Nagan Raya, Aceh, Indonesia. The condition of the peatlands which should be beneficial for the *S. rolfsii* fungus, its activity does not cause significant severity. Disease severity in susceptible varieties does not exceed 35%, and resistant varieties are only in the range of 9% [8]. Another factor that is suspected as the cause of the suppression of *S. rolfsii* is the presence of biotic component, namely the presence of other organisms that have the same environmental suitability that can suppress the activity and pathogenicity of *S. rolfsii* on peat soils.

This research is essential to carry out because there is no information available about the presence or absence of antagonistic fungi against *S. rolfsii*, the cause of peanut stem rot on peat soil Kuala Pesisir Nagan Raya. Based on the above, it is necessary to screen the fungi on the Kuala Pesisir peatlands to obtain fungi that have the potential to act as biological control agents against *S. rolfsii*, the cause of peanut stem rot.

### 2. Materials and methods

The research was conducted from September 2018 - February 2019 in Kuala Pesisir District, Nagan Raya Regency, Aceh Province, Indonesia. Isolation, morphological identification and antagonistic tests were carried out at the Plant Disease Laboratory, Faculty of Agriculture, Syiah Kuala University. The materials used were peat soil samples, pathogenic fungal isolates, namely *S. rolfsii* from peatlands, peanut seeds, potato dextrose agar (PDA), distilled water, and alcohol. The tools used were tweezers, inoculating loop, 5 mm diameter mould, measuring cup, erlenmeyer, magnetic stirrer hotplate, Petri dish, test tube, microscope, autoclave, and incubator.

The candidate fungi for biological control agents from peatlands were isolated from peanut farming in Kuala Pesisir District, Nagan Raya District, Aceh Province. Sources of inoculums were taken from three sources, namely from the weed rhizosphere around the peanut farming area (coded J), the peanut rhizosphere (coded JK), and the peanut roots (coded AK).

Isolation using the serial dilution method, from the fourth dilution 1 ml was taken and poured into a petri dish containing PDA (potatoes dextrose agar) medium, then incubated for three days at 28°C. The growing fungi were observed, then separated based on morphological differences on PDA media.

#### 2.1 Morphological identification

Fungal isolates were identified morphologically according to colour pigmentation, colony form/pattern (radial, irregular, filaments, root, shaft, etc.), texture (fibrous, cottony, smooth). Microscopically, hyphae (septate, aseptate) and conidium were observed [9].

#### 2.2 Pathogenicity test

Pathogenicity test was carried out on peanut seeds which were treated with hot water immersion and CaClO3, and then nine seeds were placed into a petri dish containing cultures of peat soil fungal isolates. Germination observations were observed on days 7 and 14. The seeds that showed necrosis and rot showed that the fungus is pathogenic. Testing of growth potency and germinability was conducted to obtain supporting data from the pathogenicity test. The growth potency was observed 14 days after inoculation with the formula [10]:

\[
GP (%) = \frac{\sum \text{seeds showing growth symptoms}}{\sum \text{planted seed}} \times 100\%
\]  

The germinability was observed based on the percentage of normal germination at count I (7 days after inoculation) dan II (14 days after inoculation) with the formula [10]:

\[
G (%) = \frac{\sum \text{normal germination count I} + \text{normal germination count II}}{\sum \text{planted seed}} \times 100\%
\]
2.3 Antagonistic test on dual culture

Pathogenic fungi were collected using a mould with a diameter of 5 mm, then grown together with the biological agent candidate fungi from peat soil. The medium used was potato dextrose agar in a 100 mm diameter Petri dish. The observation parameters are as follows:

1. Pathogen mycelium radial (mm), observation of pathogen mycelium radial is conducted to determine the presence of inhibition by candidate fungi for biological control agents from peat soil.
2. Percentage of inhibition (%). The rate of inhibition of *S. rolfsii* mycelium by the candidate antagonist agent was calculated using the formula [11] namely H = 100 x (r1 - r2)/r2. H = percentage of inhibition of *S. rolfsii* by antagonistic fungi, r1 = radial of fungal colony *S. rolfsii* that grows in the opposite direction to the antagonistic fungi and r2 = radius of *S. rolfsii* fungal colonies growing in line with antagonistic fungi.
3. Interaction Type. Observation types of interactions between pathogenic fungi with antagonistic fungi candidate performed the following categories [12]:

4. Zone of inhibition distance (mm). The inhibition zone distance is measured if there is an interaction in category C or D on the types of interactions stated above.

2.4 Molecular identification

Molecular identification was carried out by PT Genetika Science Indonesia, including PCR analysis, DNA sequences, NCBI data BLAST, and phylogenetic trees.

3. Results and discussion

The results of isolation from three isolate sources, namely the rhizosphere of weeds around peanut farm, peanut rhizosphere and peanut roots, obtained 46 fungal colonies from peat soil Kuala Pesisir Nagan Raya. Isolates that have morphological similarities with other isolates are eliminated, except those that have similarities with biological control agents are still used, with the hope that different types of isolates will be found even though their morphological appearance is the same. Pathogen isolates (*S. rolfsii*) were also not included in the observation. After similar isolates and pathogen isolates were eliminated, 22 fungal isolates were observed for the next stage.

3.1 Morphological characteristics

Morphological characters of fungal colonies on peatlands are generally green, white, greenish-white, black, and purplish-white. The surface texture is dominated by a cottony texture, while others are smooth. Colonies are mostly radial and concentric. On observation under the microscope, most of the conidium conidiophores and some in the form of sporangiophores and chlamydomspores, all hyphae were septate, and only one was aseptate (Table 1).
The fungal colonies obtained from the rhizosphere of weeds around the peanut farm were mostly green, with a cottony and smooth texture, concentric radial distribution pattern, colony forms are concentric radial. The conidium is conidiophores, and the hyphae are a septate. The same was also found in fungal colonies obtained in the peanut rhizosphere. A few other colonies obtained from the rhizosphere of weeds around the peanut farm are white, black and greenish-white. The texture is cottony and smooth, and colony form is radial, colony form is radial, conidium form is sporangiophores and chlamydospores, hyphae are septate and aseptate.

**Table 1.** Morphological characteristics of isolated fungi from peatlands.

| Isolate | Colour        | Texture   | Colony form        | Asexual reproductive | Hyphae |
|---------|---------------|-----------|--------------------|----------------------|--------|
| J1      | green         | cottony   | concentric radials| conidiophores        | septate|
| J2      | green         | cottony   | concentric radials| conidiophores        | septate|
| J3      | green         | cottony   | concentric radials| conidiophores        | septate|
| J4      | green         | cottony   | concentric radials| conidiophores        | septate|
| J5      | green         | cottony   | concentric radials| conidiophores        | septate|
| J6      | green         | cottony   | concentric radials| conidiophores        | septate|
| J7      | green         | smooth    | radial             | conidiophores        | septate|
| J8      | green         | cottony   | concentric radials| conidiophores        | septate|
| J9      | white         | cottony   | radial             | sporangiophores      | septate|
| J10     | black         | smooth    | radial             | chlamydospores       | aseptate|
| JK1     | green         | cottony   | concentric radials| conidiophores        | septate|
| JK2     | Greenish-white| smooth    | radial             | conidiophores        | Septate|
| JK3     | green         | cottony   | concentric radials| conidiophores        | Septate|
| JK4     | green         | cottony   | concentric radials| conidiophores        | Septate|
| JK5     | green         | cottony   | concentric radials| conidiophores        | Septate|
| JK6     | green         | cottony   | concentric radials| conidiophores        | Septate|
| JK7     | green         | cottony   | concentric radials| conidiophores        | Septate|
| JK8     | green         | cottony   | concentric radials| conidiophores        | Septate|
| AK1     | white         | smooth    | radial             | conidiophores        | Septate|
| AK2     | white         | smooth    | radial             | conidiophores        | Septate|
| AK3     | green         | cottony   | concentric radials| conidiophores        | Septate|
| AK4     | purplish-white| cottony   | radial             | conidiophores        | Septate|

Fungal colonies found in peanut roots had different morphological characters from colonies in the root zone of peanuts and weeds around the peanut farm. Fungal colonies on peanut roots were mostly found in white, green and purplish-white, the surface of the colonies was smooth and cottony, the distribution patterns were radial and radial concentric, the conidium forms were conidiophores and insulated hyphae.

### 3.2 Pathogenicity in dual culture

The results of pathogenicity testing on groundnut seeds found three pathogenic fungi which were indicated by the presence of necrosis of germinated groundnut seeds. The fungi are J10, AK2, and AK4, while the peanut seeds that grow on other fungal isolates are non-pathogenic. The highest growth potency was found in J3, J9, JK1, JK2, and JK8 which were not statistically significant with other non-pathogenic fungal isolates. The highest germination capacity was found in fungal isolates J3, JK1, then JK2, and JK8 and it was not significant with other non-pathogenic fungal isolates. Pathogenic fungi have the lowest potential for growth and germination compared to non-pathogenic fungi isolates (Table 2).
Overall, groundnut seeds in fungal isolates from peatland isolation, namely J3, JK1, JK2 and JK8, grew well with a potential to grow higher than 96%, and germination capacity higher than 88%. The four fungi can coexist with peanut plants and do not interfere with the germination and growth of peanut seeds. Isolate J9 had the highest growth potency; however, it was not suitable for germinability.

3.3 Antagonistic test in dual culture
The antagonistic test showed that there are fungi that have potential as biological control agents in the peatlands of Kuala Pesisir Nagan Raya. The pathogen fungi (J10, AK2, and AK4) were not included in this test, except for *S. rolfsii* as control. The smallest *S. rolfsii* mycelium radial is in J3, JK1 and JK8, while the largest are J7, J9, and AK1. The highest percentage of inhibition was found in J3, JK1 and JK8, while the lowest rate of inhibition was J7, J9, and AK1 (Table 2).

| Isolate | Pathogenicity test of peanut seeds | Antagonistic test in dual culture |
|---------|-----------------------------------|----------------------------------|
|         | Pathogenicity (%)                  | R (mm)  | PI (%) | Z (mm) | T* |
| Control | + 0.00 a                          | 57.39 H | 0.00 a | 0.00 a | 0 |
| J1      | - 88.89 bcd                       | 36.32 Abc | 36.74 | 2.20 | D |
| J2      | - 85.19 bcd                       | 39.92 Cd | 30.28 | 1.08 C |
| J3      | - 96.30 d                         | 32.60 A | 43.07 | 3.32 D |
| J4      | - 88.89 bcd                       | 33.93 Ab | 40.53 | 1.70 C |
| J5      | - 81.48 bcd                       | 35.85 Abc | 37.30 | 2.24 D |
| J6      | - 85.19 bcd                       | 42.19 D | 26.45 | f | 1.02 C |
| J7      | - 88.89 bcd                       | 56.73 H | 1.12 ab | 0.00 B2 |
| J8      | - 92.59 cd                        | 51.02 Fg | 11.04 d | 0.00 A |
| J9      | - 100.00 d                        | 54.76 Gh | 4.50 c | 0.00 B2 |
| J10     | + 3.70 a                          | - | - | - |
| JK1     | - 100.00 d                        | 33.71 A | 41.42 hi | 2.84 D |
| JK2     | - 100.00 d                        | 47.61 Ef | 16.87 de | 0.00 B1 |
| JK3     | - 88.89 bcd                       | 35.01 Abc | 38.84 | 2.32 D |
| JK4     | - 77.78 bcd                       | 44.13 De | 22.96 ef | 1.82 C |
| JK5     | - 77.78 bcd                       | 34.99 Abc | 38.85 | 1.45 C |
| JK6     | - 81.48 bcd                       | 39.51 Bcd | 31.04 fgh | 2.36 D |
| JK7     | - 85.19 bcd                       | 34.18 Abc | 40.27 gh | 1.88 C |
| JK8     | - 96.30 d                        | 31.71 A | 44.71 i | 2.54 D |
| AK1     | - 85.19 bcd                       | 55.81 Gh | 2.71 bc | 0.00 B2 |
| AK2     | + 62.96 b                         | - | - | - |
| AK3     | + 88.89 bcd                       | 34.36 Abc | 40.02 ghi | 0.00 B2 |
| AK4     | + 66.67 bc                        | - | - | - |

Table 2. Pathogenicity and antagonistic tests of fungi isolated from peat soil in Kuala Pesisir Nagan Raya.

| Isolate | Pathogenicity test of peanut seeds | Antagonistic test in dual culture |
|---------|-----------------------------------|----------------------------------|
|         | Pathogenicity (%)                  | R (mm)  | PI (%) | Z (mm) | T* |
| Control | + 0.00 a                          | 57.39 H | 0.00 a | 0.00 a | 0 |
| J1      | - 88.89 bcd                       | 36.32 Abc | 36.74 | 2.20 | D |
| J2      | - 85.19 bcd                       | 39.92 Cd | 30.28 | 1.08 C |
| J3      | - 96.30 d                         | 32.60 A | 43.07 | 3.32 D |
| J4      | - 88.89 bcd                       | 33.93 Ab | 40.53 | 1.70 C |
| J5      | - 81.48 bcd                       | 35.85 Abc | 37.30 | 2.24 D |
| J6      | - 85.19 bcd                       | 42.19 D | 26.45 | f | 1.02 C |
| J7      | - 88.89 bcd                       | 56.73 H | 1.12 ab | 0.00 B2 |
| J8      | - 92.59 cd                        | 51.02 Fg | 11.04 d | 0.00 A |
| J9      | - 100.00 d                        | 54.76 Gh | 4.50 c | 0.00 B2 |
| J10     | + 3.70 a                          | - | - | - |
| JK1     | - 100.00 d                        | 33.71 A | 41.42 hi | 2.84 D |
| JK2     | - 100.00 d                        | 47.61 Ef | 16.87 de | 0.00 B1 |
| JK3     | - 88.89 bcd                       | 35.01 Abc | 38.84 | 2.32 D |
| JK4     | - 77.78 bcd                       | 44.13 De | 22.96 ef | 1.82 C |
| JK5     | - 77.78 bcd                       | 34.99 Abc | 38.85 | 1.45 C |
| JK6     | - 81.48 bcd                       | 39.51 Bcd | 31.04 fgh | 2.36 D |
| JK7     | - 85.19 bcd                       | 34.18 Abc | 40.27 gh | 1.88 C |
| JK8     | - 96.30 d                        | 31.71 A | 44.71 i | 2.54 D |
| AK1     | - 85.19 bcd                       | 55.81 Gh | 2.71 bc | 0.00 B2 |
| AK2     | + 62.96 b                         | - | - | - |
| AK3     | + 88.89 bcd                       | 34.36 Abc | 40.02 ghi | 0.00 B2 |
| AK4     | + 66.67 bc                        | - | - | - |

Statistical analysis using a completely randomised design. The numbers followed by the same letter in the same column are not significantly different at the 5% level (Duncan’s test).
P: Pathogenicity, GP: Growth potency, G: germinability, R: Pathogen radial, PI: Percentage of inhibition, Z: Inhibition zone, and T: Interaction type.
(*) Category refers to figure 1.

The fungi colonies of J3, JK1, and JK8 have potential as antagonistic fungi for *S. rolfsii*. That is because this fungus can suppress pathogens in the dual culture test. This fungus has a high inhibiting ability which makes it difficult for pathogens to grow in the media. The low pathogenic mycelium radial indicates this in the J3, JK1 and JK8 colonies, namely 32.6 mm, 33.71 mm and 31.71 mm, as well as the percentage of inhibition of the three fungi, namely 43.07%, 41.42. % and 44.71%. The three fungi
also have a large inhibition zone with an inhibition zone distance of more than 2.5 mm, and this fungus is classified into type D.

![Figure 2. The interaction of pathogens and fungi as candidates for biological control of peatlands in a dual culture test.](image)

### 3.4 Molecular identification

The PCR results of the product (Figure 3) showed that the peatland fungal isolates, which had the potential to be antagonistic agents, had DNA fragments length of 574 - 584 bp (Table 3). From the DNA sequencing results, the top 10 hits BLAST were obtained from the NCBI data. From these data, it was continued to the phylogenetic tree stage using a neighbour-joining tree. These results indicate that the selected fungal isolates are related to *Trichoderma asperellum*.

![Figure 3. Gel photo - PCR product of fungal isolate potential as a biological control agent of *S. rolfsii* in peat soil.](image)

Table 3. Results of fungal DNA sequencing from peatland isolates which have the potential to be biological control agents.

| No | Isolate code | Species                  | Accession           | DNA fragment length |
|----|--------------|--------------------------|---------------------|--------------------|
| 1  | J3           | *Trichoderma asperellum* | MK211208.1          | 574 bp             |
| 2  | JK1          | *Trichoderma asperellum* | MK211208.1          | 574 bp             |
| 3  | JK8          | *Trichoderma asperellum* | MK210428.1          | 584 bp             |

Trichoderma fungi can overgrow so that they can compete for space and nutrition with plant pathogenic fungi [13]. Apart from doing a competition, the fungus *Trichoderma asperellum* also carries
out mycoparasites which can harm pathogens. This parasitism is necrotrophic so that the pathogenic hyphae cannot be recovered from damage [14]. The fungus *T. asperellum* conducts mycoparasites against *S. rolfsii* by penetrating the walls of the pathogen hyphae. *Trichoderma asperellum* produces protease and β-1,3-glucanase enzymes in large amounts and chitinase enzymes in smaller quantities, these enzymes are useful in cell wall degradation. This fungus also has secondary metabolite compounds of peptaibol, namely Trichovirin, Trichotoxin, Trichorzin, and Hypomurocin. Other secondary metabolite compounds are polyketones (pyrone), terpenes, and carboxylic acids and their derivatives [15].

![Phylogenetic tree of peatland antagonistic fungal isolates using the neighbor joining tree by NCBI blast tree method.](image)

Peatlands are one of the natural habitats for Trichoderma, including *Trichoderma asperellum*. The results showed that in peatlands such as in Kalimantan, including Sarawak, many Trichoderma fungi were found. Trichoderma types such as *Trichoderma asperellum* are found in the peatlands of Kalimantan and Sarawak, Malaysia [16]. Other areas in Sarawak are also found in Alan Bunga, namely
secondary forest, logged-over secondary forest and oil palm planted areas [17]. Trichoderma fungi such as *Trichoderma harzianum* are also commonly found in the peatlands of Kalimantan, especially in areas of grove forest and plant rhizosphere, Another *Trichoderma* species that is often found is *Trichoderma asperellum* [18, 19].

4. Conclusion

The results of the pathogenicity and antagonistic test concluded that the Kuala Pesisir - Nagan Raya peatlands had antagonistic fungi that had the potential to be biological control agents against *Sclerotium rolfsii*, namely isolates J3, JK1 and JK8. The results of identification both morphologically and molecularly showed that the fungal isolates J3, JK1, and JK8 were related to *Trichoderma asperellum*.

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