The potential of soil fungi associated with potato rhizosphere to control root knot nematode (*Meloidogyne spp.*) on potato

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Abstract. The root knot nematode (*Meloidogyne spp.*) is one of important pathogens on potato crops in North Sumatra, Indonesia. This nematode causes significant crop losses on potato directly and indirectly. The effect of fungal isolates (*Trichoderma* sp. 1, *Mucor* sp.1, *Aspergillus* sp. 2, *Mucor* sp. 2) that were isolated from rhizosphere of potato in North Sumatra were studied in green house experiments on the growth of potato and the reproduction of the nematode (*Meloidogyne spp*). The results showed that *Trichoderma* sp. 1 caused a significant gall reduction, while *Mucor* sp.1 and *Mucor* sp.2 could improve the growth of potato.

1. Introduction
North Sumatra is one of the potato production centres in Indonesia. The data showed that potato productions in North Sumatera in 2012 was 128,965 tons, more than the production in 2011, which was 123,078 tons [1]. However, the results of previous survey and research found that most locations of the potato farming in North Sumatera have been infested by root knot nematode (*Meloidogyne spp./RKN*). Moreover, more than one RKN species is found in one location in several places [2]. Three RKN species, namely *Meloidogyne arenaria*, *Meloidogyne incognita*, and *Meloidogyne javanica*, were found at the potato farming in Karo District, North Sumatera [2].

Some strategies to control nematode have been reported, including cultural practices, plant resistance, and nematicide, but they have not given the satisfying result yet. The use of nematicide for controlling nematode gives the negative impact to the quality of environment, the balance of ecosystem and the human health. The use of nematode-resistant crop varieties are effective and economical way in controlling the plant parasite nematodes. Besides it can reduce the nematode population and the application is easily implemented on the field, this technique can also reduce the cost production to the lowest level. Unfortunately, potato resistance to *Meloidogyne spp.* is not available yet at the agricultural stores in North Sumatera.

Biological control with fungi which associates with potato plant rhizosphere, to control the parasite nematode especially the root knot nematode is the potential alternative technology to be developed. This is because fungi are the organisms that are naturally existed in the nature and have the same habitat with the plant parasite nematode, not dangerous to environment, easily multiplied on artificial medias with low cost, easily applied and will develop naturally and can survive when there is no host nematode, so it will become saprophyte inside the soil [3]. The aim of this study was to observe the
fungi potential of the potato plant rhizosphere in controlling the population of root knot nematode in the green house.

2. Material and methods

2.1. The evaluation in the greenhouse

The evaluation was conducted in the greenhouse of Balai Penelitian Tanaman Buah (BPTB), Tongkoh, Brastagi district by using potato G0 cultivar Granola. Each potato tuber was planted in polybag with the size of 25 cm x 25 cm that contained the mixture of topsoil, sand and fertilizer (2 : 1 : 1 / w / v) (5 kg / polybag). The polybags are arranged based on Complete Randomized Design in the green house. The plants were taken care of for 12 weeks. The moisture of sand inside each polybag was kept with daily watering.

2.2. The evaluation of antagonistic fungal isolates

Based on in vitro screening, four antagonistic fungi isolates were selected and used for the evaluation in the greenhouse. They were Trichoderma sp1. (A1), Mucor sp1. (A2), Aspergillus sp2. (A3), and Mucor sp2. (A4). All fungi isolates were first cultured on Potato Dextrose Agar (PDA) on petri plates. The plates were incubated at 24˚C for 14 days. The produced conidia were collected from cooked rice. 30 gr/polybag from each fungi (10⁶ spores/g cooked rice [4], which had been counted with hemocytometer [5], were applied separately into each polybag. At the same time 10 ml suspension that contained 500 juveniles Meloidogyne spp. were applied into each polybag. The control treatments, which were the plants that were inoculated with only Meloidogyne spp. (A0), and only nematicide (Carbofuran 3%) (A5). Each treatment was conducted with 4 replications. Plants were fertilized by using NPK fertilizer (1 g/l liter water) if it was needed. The observation was conducted to plant height at 1 – 12 weeks after application, tuber weight, root fresh weight and the number of root knot [6] at the end of evaluation (12 weeks after the application).

2.3 Statistical analysis

Data was statistically analyzed according to standard analysis of variance by a one way ANOVA with SPSS software. The comparison between means was carried out by Duncan’s Multiple Range Test [7] with level of 5%.

3. Results and discussion

The results showed that four fungal isolates, gave the significant influence in increasing the addition of height of potato plants compared with the control treatment and nematicide (Carbofuran 3%) (Table 1). The highest plant height was at the treatment of Mucor sp 1 (19.48 cm). This result was not significantly different with Mucor sp.2 (19.10 cm), Trichoderma sp.1 (18.31 cm), and Aspergillus sp.2 (17.94 cm). Similar results were obtained on the observations of tuber weight and root fresh weight. All 4 fungal isolates gave the better result compared with control and the giving of nematicide (Carbofuran 3%) (Table 2).

The evaluation result indicated that the antagonistic fungal application including Trichoderma, Mucor dan Aspergillus could increase the plant growth like the plant height, root fresh weight, tuber weight and reduce the number of root knot. This result proved that the secondary metabolite activity produced by antagonistic fungi had nematicidal effect, therefore it affected the existence of nematode inside the root. Nematode could not develop well inside the root [8, 9, 10] (Figure 1). Trichoderma sp. produces volatile compound, i.e. 6-pentyl-a-pyrene (6-PAP), 1-bvinylcyclopentane-1a, 6-pentyl-2H-pyran2-one, and 4-(2-hydroxyethyl) phenol that had nematicidal effect [11]. Furthermore, the attacked root would die and caused the plant growth inhabit [11]. The response of the plant to the root knot nematode was the response from all parts of the plant and response from the cells of the plant, all parts of the plant gave response to infection, and the decrease rate of photosynthesis, growth and production.
In contrast, the application of nematicide (Carbofuran 3%), did not give the significant difference with the control treatment. On this treatment, the plant height, root fresh weight, lower tuber weight were obtained, meanwhile the number of root knot were higher compared with the treated plants with the antagonistic fungi. This result indicated that nematicide (Carbofuran 3%) was resistant to control root knot nematode. This type of nematicide (Carbofuran 3%) is usually applied by the farmers in controlling nematode.

Figure 1. The symptoms of potato root infected by root knot nematode (Meloidogne sp.): A. Galls on potato root, B. Female nematode, C. Nematode egg mass (arrows)

Table 1. The effects of antagonistic fungal application to potato plant

| Treatments                  | Plant height (cm) | Time of observation (weeks after application) |
|-----------------------------|-------------------|-----------------------------------------------|
|                             | 1            | 2       | 3       | 4       | 5       | 6       | 7       | 8       | 9       | 10      | 11      | 12      |
| Control (A0)                | 0.00         | 0.00    | 0.38    | 0.79    | 3.49    | 4.28    | a       | 6.80    | a       | 9.44    | a       | 10.54   | a       | 11.69   | a       | 11.76   | a       |
| Trichoderma sp. 1 (A1)      | 0.00         | 0.00    | 0.00    | 1.61    | 5.10    | 9.06    | b       | 12.50   | b       | 16.35   | b       | 16.68   | b       | 18.31   | b       | 18.31   | b       |
| Mucor sp. 1 (A2)            | 0.00         | 0.61    | 0.43    | 1.86    | 6.68    | 9.90    | b       | 12.63   | b       | 16.44   | b       | 16.69   | b       | 19.44   | b       | 19.44   | b       |
| Aspergillus sp. 2 (A3)      | 0.00         | 0.13    | 0.63    | 5.75    | 8.98    | 12.54   | b       | 15.06   | b       | 16.00   | b       | 17.94   | b       | 17.94   | b       | 17.94   | b       |
| Mucor sp. 2 (A4) Nematicide (Carbofuran 3%) (A5) | 0.00 | 0.68 | 0.38 | 1.81 | 6.61 | 9.46 | b | 12.55 | b | 16.43 | b | 16.46 | b | 19.06 | b | 19.06 | b | 19.10 | b |

Notes: The numbers followed by the same letter notations in the same table are not significantly different on Duncan’s multiple range test with level of 5 %

Table 2. The effects of antagonistic fungi application to tuber weight, root fresh weight, and number of root knot on potato

| Treatments                  | Tuber weight (g) | Root fresh weight (g) | Number of galls |
|-----------------------------|------------------|-----------------------|-----------------|
| Control (A0)                | 77.9 a           | 0.19 b                | 41.75 b         |
| Trichoderma sp. 1 (A1)      | 24.31 bc         | 1.61 a                | 19.13 a         |
| Mucor sp. 1 (A2)            | 30.98 c          | 1.88 b                | 36.00 b         |
| Aspergillus sp. 2 (A3)      | 20.35 bc         | 2.09 b                | 38.50 b         |
| Mucor sp. 2 (A4)            | 31.09 c          | 2.82 b                | 54.13 bc        |
| Nematicide (Carbofuran 3%) (A5) | 13.29 ab | 0.35 a | 67.25 c |

Notes: The numbers followed by the same letter notations in the same table are not significantly different on Duncan’s multiple range test with level of 5 %
These results convince us that antagonistic fungi give the direct or indirect effects to the reproduction of nematode (number of root knot) and the response of host (plant growth). All antagonistic fungi that are obtained from the rhizosphere of potato plants that are used on this research have potentials as the biocontrol agents and as the biological fertilizer to control Meloidogyne spp. on potato plants under the condition inside the greenhouse.

4. Conclusions
In greenhouse trial, Trichoderma sp. 1 gave significat effect as antagonistic agents, which caused lower number of galls compared with than the other fungi. Mucor sp. 1 and Mucor sp. 2 gave effect as the biological fertilizer because both isolated increased the plant height, tuber weight and root fresh weight.

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