Arthrobotrys thaumasia and A. musiformis as biocontrol agents against Meloidogyne hapla on tomato plant

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Abstract. Purba RTT, Ducci F, Sari RW, Naibaho DC, Putri QA, Maulana A, Hastutai LDS, Punnayak H. 2022. Arthrobotrys thaumasia and A. musiformis as biocontrol agents against Meloidogyne hapla on tomato plant. Biodiversitas 23: 3659-3666. The use of nematode-trapping fungi as a biocontrol agent is an environmental-friendly step in controlling Meloidogyne hapla on plants. The aim of this study was to determine the potential of A. thaumasia and A. musiformis as biocontrol agents in reducing the attack of M. hapla on tomatoes (Solanum lycopersicum). The isolates were tested for their biocontrol ability against M. hapla using a completely randomized design (CRD) with five treatments: K+ (positive control), K- (negative control), KBF (Carbofuran), and two treatments of nematode-trapping fungi (A. thaumasia and A. musiformis). Root length, root weight, stem length, and stem weight of tomato plants were observed. The results showed that A. thaumasia and A. musiformis could reduce M. hapla in tomato plants up to 93% and 97%, respectively. Number of root-knot infections caused by M. hapla in tomato was found lower in JPN2 treatment, followed by JPN1 treatment. It was also recorded that JPN2 isolate treatment was able to produce the highest values of root length, root wet and dry weight, stem length, and stem wet and dry weight as compared to JPN1 isolate treatment.

Keywords: Biological control, carbofuran, nematode-trapping fungi, plant growth, root-knot nematode

Abbreviations: NTF: nematode-trapping fungi; PPN: plant pest nematodes; RKN: root-knot nematode

INTRODUCTION

The global progressive growth of the human population has been making greater demand of global food supply which further gives greater challenges in food management and security. Safe agricultural crop management must be included in prioritization, nowadays pathogens are susceptible to various pests such as bacteria, fungi, viruses and other microorganisms. An important crop pest plants pest nematodes (PPN), which attack a wide range of agricultural crops, including root-knot nematodes, cyst nematodes, root-lesion nematodes, virus-vector nematodes, etc. (Ahmad et al. 2021).

Root-knot nematodes refer to a group of microscopic and parasitic nematodes that frequently infect crop plants such as tomato, potato, banana, tobacco and other important vegetables. They mainly belong to the genus Meloidogyne, especially M. incognita, M. hapla, M. arenaria, and M. javanica. It is estimated that root-knot nematodes cause 5% or 78 billion dollars annually in crop yield losses worldwide (Ralmi et al. 2016; Ahmad et al. 2021).

Meloidogyne hapla is one of root-knot nematode (RKN) that is very difficult to control because it attacks more than 2000 plant species and causes yield losses in tropical and sub-tropical agriculture, especially horticultural crops such as tomato (Solanum lycopersicum) with varying degrees of damage and very detrimental to the economy (Indarti and Rahayu TP 2014; Yus et al. 2014; Huang et al. 2016; Winarto et al. 2018). M. hapla attacks on plants are characterized by the presence of root cavities. The second-stage larvae produce wall-degrading cell enzymes such as cellulases, pectate lyases, and endoxygenases. They then insert these enzymes into host cells behind the elongation zone of the root tip. Eventually, the enzymes travel upwards to find a differentiation region with vascular cells and form a feeding site (Ahmad et al. 2021). Due to the appearance of root knots on plants, the roots of the plant swell and are not able to function properly. This triggers a disruption in plant metabolic processes and can make plants more susceptible to other diseases caused by viruses, bacteria, and fungi that further increase the risk of plant death (Dutta et al. 2012; Liu et al. 2019; Khan et al. 2020).

Carbofuran and other chemical nematodes are commonly used to control root-knot nematodes in tomato plants (Ralmi et al. 2016; Ahmad et al. 2021). However, the continuous and inappropriate use of carbofuran-containing toxic chemicals causes environmental damage, death of non-target organisms around agricultural land and even human health (Hahn et al. 2019). The use of biological agents such as nematode-trapping fungi (NTF), besides bacteria, fungi and other microbes, is a more appropriate and environmentally friendly way of
controlling root-knot nematodes, which can be used to suppress the environmental spread of this parasitic nematode infection in vegetable crop since it has extremely low intervention against ecological food chain rather than chemical substances (Honghong et al. 2016; Ahmad et al. 2021). NTF is a group of microscopic fungi that produce their trapping hyphae (adhesive or non-adhesive traps) to catch and destroy nematodes, like parasitic or saprophyte nematodes (Hsueh et al. 2013; Poveda et al. 2020). NTF such as Arthrobotrys oligospora, Candelabrella musiformis, Dactylaria eudermata, and Monacrosporium eudermatum are known to significantly suppress M. incognita attacks on crop plants (Hastuti and Faull 2018; Soliman et al. 2021). It also was reported that several species of NTF are able to suppress M. hapla (Hussain et al. 2016). The objectives of the study were to observe the ability of two NTF isolates, namely Arthrobotrys thaumasia and Arthrobotrys musiformis isolated from Deli Serdang in suppressing M. hapla population on infected tomato plants compared to plants treated with carbofuran. The correlation between the isolates existence and plant growth was observed by calculating root length, root wet and dry weight, stem length, stem wet and dry weight.

MATERIALS AND METHODS

Procedures

Fungal culturing and preparation of conidia suspension

Isolates of two nematode-trapping fungi, namely Arthrobotrys thaumasia and A. musiformis from Deli Serdang Regency were cultured on potato dextrose agar (PDA) medium at 25°C for 14 days. The petri dishes were observed visually and then through a microscope at 100x magnification.

Conidial suspension was prepared by taking five fungal agar discs of 5 mm using a cork borer from each fungal isolate. Five discs of each fungal isolate were put into a sterile centrifuge tube containing 10 mL of 0.05% Tween 80 solution. The centrifuge tube was homogenized using a vortex for 1 minute to release the conidia. Conidial density was calculated using a hemocytometer with the aid of a microscope (400x) and the conidial concentration was adjusted to 107 conidia/mL. 107 conidia/mL conidial suspension was sprayed into each polybag of experimental plants (Hastuti and Faull 2018).

Preparation of tomato plant growing media

Tomato plants were grown in compost soil. The composted soil was dried and then sterilized by autoclaving at 121°C temperature for ±60 minutes. The sterilized compost was transferred to polybags with a diameter of 20 cm and filled with 300 g of sterile soil. To prevent contamination, the polybag was covered with aluminum foil on the top and bottom surfaces of the polybag before use. Experimental polybags treated with nematode-trapping fungal isolates were given 10 mL of fungal suspension containing 107 conidia/mL. Polybags are then stored in a room with a temperature of 25°C for 7 days before planting tomato seeds (Hastuti and Faull 2018).

Preparation of comparative chemical agent

Carbofuran was used as chemical control for root-knot nematode control. As much as 2 mg of carbofuran was crushed and homogenized with 10 ml of methanol. 10 mL of the solution was then poured into polybags with carbofuran treatment. Polybags were then stored in a room with a temperature of 25°C for 7 days before planting tomato seeds (Hastuti and Faull 2018).

Bioassay test of nematode-trapping fungi against Meloidogyne hapla on tomato plants

The experiment was done in a completely randomized design (CRD). Bioassay test was conducted according to (Hastuti and Faull 2018) with modifications. Tomato (S. lycopersicium) seeds were sown for 30 days. After 30 days, tomato seedlings were transferred to polybags filled with compost soil and the mixture was mixed according to the treatment. Experimental polybags were divided into five treatment groups: (i) positive control (K+): polybags of tomato plants that were treated with only M. hapla; (ii) negative control (K-): polybags of tomato plants without M. hapla and without any NTF isolates; (iii) carbofuran (KBF): polybag of tomato plants containing M. hapla treated with carbofuran; (iv) JPN1: polybag of tomato plants containing M. hapla treated with carbofuran; (v) JPN2: polybag of tomato plants containing M. hapla treated with A. musiformis.

All polybags were incubated for seven days and then tomato plants were transferred to test polybags. M. hapla used was in juvenile phase 2 and approximately 1000 individuals were added to each treatment except control (K-). Four replicates of each treatment were observed for 3 different intervals, i.e., 7, 14, and 30 days after the seven-day addition of M. hapla.

Data analysis

Observations were made at three different intervals to see the effect of M. hapla infection on the growth of tomato plants. To count the number of infections in the root of tomato plants, the root border was cut from the stem and then washed with water. After that, the roots were soaked with phloxine B dye and observed the cavities on the roots. The number of vermiciform nematodes was counted on the roots on 7th day. On 14th day, the swelling was observed on the root caused by adult female nematodes. On the 30th day, a number of root cavities were counted containing adult female nematodes that produced eggs.

The effect of M. hapla infection on tomato plant growth was noted by calculating the wet weight and dry weight of roots and stems, root length and stem height in each experimental polybag. The results obtained were analyzed by ANOVA using SPSS version 21.00 (Hastuti and Faull 2018).

RESULTS AND DISCUSSION

Morphological observation of NTF isolates

Two isolates of nematode-trapping fungi, namely A. thaumasia and A. musiformis, were cultured on PDA and visualized with a light microscope. Morphological view of both the fungi was presented in Figures 1 and 2.
Figure 1. A. 14 days old colony of *Arthrobotrys thaumasia* on PDA; B. conidia (arrow a) and (arrow b) conidiophore colored with lactophenol cotton blue; C. non-septate micro conidia; D. contractile-ring-like trap hyphae; E. presence of nematode (arrow b) trapped by NTF hyphae (arrow a)

Figure 2. A. 14 days old colony of *Arthrobotrys musiformis* on PDA; B. hyphae (arrow a), (arrow b) conidiophore colored with lactophenol cotton blue and (arrow c) conidia; C. conidia; D. sticky-web-like trap hyphae; E. a nematode (arrow b) trapped by NTF hyphae (arrow a)
Bioassay test of NTF against *M. hapla* infection on tomato plants (*Solanum lycopersicum*)

**Day 7**

On day 7, *M. hapla* infection was noted in tomato plants by counting the number of *M. hapla*, which began to enter the root tissue of tomato plants (vermiform). The results showed the JPN1 and JPN2 treatments had a lower number of vermiform *M. hapla* compared to the K+ and KBF treatments (Figure 3).

The results of the statistical test showed that there was no significant difference between each treatment (Figure 3). However, it was noted that the treatment using isolates of *A. thaumasia* and *A. musiformis* was able to suppress *M. hapla* attack on the roots as a small amount of vermiform was found in plant root tissue. The highest amount of vermiform was found in K+ treatment and the least in JPN1 treatment, followed by JPN2 treatment. The lower amount of vermiform found in the roots of JPN1 and JPN2 treatments indicated that nematode-trapping fungi were able to suppress *M. hapla* infection in tomato plant roots.

**Day 14**

On day 14 *M. hapla* infection was observed on tomato plant roots by counting and observing root swelling caused by adult female nematodes that were ready to lay eggs. The results of 14th day can be seen in Figure 4.

Based on Figure 4, JPN2 treatment using *A. musiformis* isolate was most effective in suppressing *M. hapla* infection because there was no root swelling caused by adult female nematodes ready to lay eggs, followed by JPN1 treatment using *A. thaumasia* isolate. The attack and presence of the female nematode *M. hapla* on tomato plant roots were highest in K+ treatment, followed by KBF treatment. The results of the statistical test also showed that there was a significant difference between each treatment.

The lower number of root swelling caused by adult female nematodes in JPN1 and JPN2 treatments was influenced by the ability of fungi to trap nematodes from fungal hyphae. *A. thaumasia* and *A. musiformis* were known to form trap structures after 24 hours of injection of *M. hapla* in the test plants. *A. musiformis* forms single ring-shaped traps or adhesive webs and *A. thaumasia* also forms single contraction ring traps, which were formed due to external stimuli provided by nematodes to induce trap formation.

**Day 30**

The number of root nodules on tomato roots caused by adult female *M. hapla* laying eggs on tomato root tissue was observed on 30th day. Treated plants (addition of nematode-trapping fungi) had a higher ability to suppress the growth of nematodes on plant roots, the results of which can be seen in Figure 5.

The results of statistical tests exhibited that there was a significant difference between each treatment (Figure 5). The best treatment in suppressing nematode attack on 30th day in terms of the number of root cavities was shown by JPN2, followed by JPN1, and KBF treatment. The highest number of root cavities was found in K+ treatment, while K- treatment had no root cavities. JPN1 and JPN2 treatments were more effective in suppressing *M. hapla* attack on tomato roots as compared to KBF treatment using a synthetic nematicide (carbofuran).

**Figure 3.** The number of vermiform *Meloidogyne hapla* on tomato roots on 3rd day. (K+ treatment with *M. hapla*; K- without treatment; KBF treatment with *M. hapla* and carbofuran; JPN1 treatment with *M. hapla* and *Arthrobotrys thaumasia*; JPN2 treatment with *M. hapla* and *A. musiformis* isolate)

**Figure 4.** The amount of root swelling caused by the adult female nematode *Meloidogyne hapla* on tomato roots on 14th day. (K+ treatment with *M. hapla*; K- without treatment; KBF treatment with *M. hapla* and carbofuran; JPN1 treatment with *M. hapla* and *Arthrobotrys thaumasia*; JPN2 treatment with *M. hapla* and *Arthrobotrys musiformis* isolate)

**Figure 5.** The amount of root swelling caused by the adult female nematode *Meloidogyne hapla* on tomato plant roots on 30th day. (K+ treatment with *M. hapla*; K- without treatment; KBF treatment with *M. hapla* and carbofuran; JPN1 treatment with *M. hapla* and *A. thaumasia*; JPN2 treatment with *M. hapla* and *A. musiformis* isolate)
Effect of *Meloidogyne hapla* infection on tomato plant growth (*Solanum lycopersicum*)

**Tomato plant root length and weight**

At 30 days of observation, K- treatment had the highest average root length, followed by JPN1 and JPN2 treatments (Figure 6). The high average number of additional root lengths of tomato plants was observed in K- treatment, which was influenced by the absence of infection by *M. hapla* with no disturbance of plant growth. JPN1 and JPN2 treatments showed a high average increase in root length at each observation time of 30 days. This can be influenced by the addition of *A. thaumasia* and *A. musiformis*, which can support plant growth so that the root cavities formed on roots of JPN1 and JPN2 treatments do not cause significant root disturbances. The same thing was also seen from the results of statistical tests on root wet weight and root dry weight of tomato plants at each observation time, where K- treatment had the highest average, followed by JPN1 and JPN2 treatments. The results of statistical tests on root wet weight and root dry weight can be seen in Figures 7 and 8.

Statistical tests on root wet weight and root dry weight showed significant results. However, in a statistical test of root wet weight, there was no significant difference between the K+, KBF, and JPN1 treatments (Figure 7). In the statistical test of root dry weight, there was a significant difference between JPN1 and JPN2 treatments and the KBF and K+ treatments (Figure 8). It can also be stated that JPN1 and JPN2 treatments showed better results in increasing the weight of plant roots and giving different treatments had an effect on the root growth of tomato plants.

**Tomato plant stem length and weight**

The results of statistical tests showed that K- treatment had the highest average root length, followed by JPN2 and JPN1 treatments (Figure 9). The highest average number of additional stem lengths of tomato plants in K- treatment was influenced by the absence of infection by *M. hapla* so that there was no growth disturbance in the length of plant stems. In addition to the K- treatment, the JPN2 and JPN1 treatments were treatments that showed a high average increase in root length at each observation time of 30 days. The same thing was also observed from the results of statistical tests on stem wet weight and stem dry weight of tomato plants at each observation time, where K- treatment had the highest average value, followed by JPN1 and JPN2 treatments. The results of statistical tests on the wet weight of the stem and the dry weight of the stem can be seen in Figures 10 and 11.

Statistical tests on stem wet weight and stem dry weight showed significant results, but there was no significant difference between K+, KBF, JPN1 and JPN2 treatments (Figures 10 and 11). However, based on the above figures, it can be stated that JPN1 and JPN2 treatments showed better results in increasing plant weight and different treatments had an effect on the growth of tomato plant stems.

**Discussion**

The bioassay test showed that *A. thaumasia* and *A. musiformis* had potential as biocontrol agents because they could suppress *M. hapla* attack for 30 days of observation by 93% (*A. thaumasia*) and 97% (*A. musiformis*), compared to treatment with carbofuran which could only suppress *M. hapla* attack by 86%. Previously *A. thaumasia* was reported to be able to reduce *M. incognita* multiplication factor *in vivo* by 80% in tomato roots (Kassam et al. 2021). Nematophagous fungus from the same genus of *A. oligospora* was also observed effectively suppressing the nematode population in tobacco roots (Hastuti and Faull 2018) and tomato roots (Soliman et al. 2021). These biocontrol agents have proven to be able to support plant growth in terms of increasing root length, root wet and dry weight, stem length, stem wet and dry weight well compared to other treatments. *A. oligospora* also supported plant growth (Hastuti and Faull 2018).
Figure 7. Wet weight of tomato roots at 7, 14 and 30 days. (K+ treatment with *Meloidogyne hapla*; K- without treatment; KBF treatment with *M. hapla* and carbofuran; JPN1 treatment with *M. hapla* and *Arthrobotrys thaumasia*; JPN2 treatment with *M. hapla* and *Arthrobotrys musiformis* isolate)

Figure 8. Dry weight of tomato roots at 7, 14 and 30 days. (K+ treatment with *Meloidogyne hapla*; K- without treatment; KBF treatment with *M. hapla* and carbofuran; JPN1 treatment with *M. hapla* and *Arthrobotrys thaumasia*; JPN2 treatment with *M. hapla* and *Arthrobotrys musiformis* isolate)

Figure 9. Stem length of tomato plant at 7, 14 and 30 days. (K+ treatment with *Meloidogyne hapla*; K- without treatment; KBF treatment with *M. hapla* and carbofuran; JPN1 treatment with *M. hapla* and *Arthrobotrys thaumasia*; JPN2 treatment with *M. hapla* and *Arthrobotrys musiformis* isolate)
According to Peiris et al. (2020), nematode-trapping fungi have various mechanisms to capture and kill nematodes, including forming trapping devices derived from fungal hyphae or nematode-trapping fungi. Nematode-trapping fungi have an excellent ability to trap and reduce the population of plant-parasitic nematodes. This ability is of great importance for use as biocontrol agents in agriculture (Moosavi and Zare 2020; Zhang et al. 2020). Hamza et al. (2018) and Li et al. (2015) also noted that these fungi produce extracellular enzyme compounds that inhibit the movement of nematodes and destroy the cuticle of the nematode, which is the part of the nematode body that it uses to penetrate the roots of the infected plant. Winarto et al. (2018) argue that the control of *M. hapla* still relies on the use of synthetic pesticides that can pollute the environment, kill natural enemies of nematodes, and are resistant to nematodes. Empowerment of biological agents as natural enemies of nematodes, such as nematode-trapping fungi, needs to be done to control nematodes. Huang et al. (2016) added that the use of nematode-trapping fungi as biocontrol agents showed better efficiency in reducing nematode attack and the combination of two types of nematode-trapping fungi showed more effective results than synthetic nematicides. It is a promising strategy as a biocontrol agent against *M. hapla* in agriculture.

According to Hastuti and Faull (2018), the use of fungal inoculants from the nematode-trapping group can increase plant growth due to an increase in auxin production caused by high microbial activity in the root area of the host plant. This affects the increase in organic and inorganic components in host plant cells and simultaneously can increase the wet weight and dry weight of the plant. Singh et al. (2013) and Marin-Bruzos and Grayston (2019) stated that nematode-trapping fungi are capable of reducing the number of pathogenic nematodes on plant roots. These fungi can also increase plant growth by supporting the improvement of other soil microflora and the production of plant secondary metabolites that can interfere with reproduction.
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