

Research Article

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Control of Root-knot Nematodes (Meloidogyne spp.) on Tomato (Lycopersicum esculentum Mill.) using Endophytic Fungi Trichoderma asperellum and Beauveria bassiana

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Abstract

Nematodes *Meloidogyne* spp. is the most important phytonematode in the world, especially in agriculture in tropical areas. The level of root damage to tomato plants caused by the nematode *Meloidogyne* spp. can reach 68.3%. *Meloidogyne* nematodes can be controlled biologically using the endophytic fungus *Trichoderma asperellum* and *Beauveria bassiana*. The aim of the research was to determine the ability of the endophytic fungi *T. asperellum* and *B. bassiana* as nematicides to control root knot nematodes *Meloidogyne* spp. on tomatoes. This study used two isolates of *T. asperellum* (SD324 and S2D11) and two isolates of *B. bassiana* (TD312 and PB211). The endophytic fungi *T. asperellum* and *B. bassiana* are applied to the soil one week before planting tomato seedlings, while nematode eggs are applied one week after planting tomato seedlings. The results of the research show that the endophytic fungi *T. asperellum* and *B. bassiana* applied to the soil can suppress the number of galls and eggs masses, eggs per egg mass of *Meloidogyne* spp. *Beauveria bassiana* TD312 is the best isolate for suppressing the development of nematode populations in tomato plants.

Keywords: biological control, endophytic fungi, galls, nematodes

1. Introduction

Tomato plants (*Lycopersicon esculentum* Mill.) are an important horticultural commodity, especially as a vegetable crop. Tomatoes contain vitamins and minerals necessary for growth and health in the body. Apart from that, tomatoes also contain carbohydrates, protein, fat, and calories (Fitriani, 2012). Mature, red tomatoes are a source of vitamin A, vitamin C, and a little vitamin B (Sunarjono, 2016).

The productivity of tomato plants in Indonesia in 2018–2020 tends to increase, respectively, to 18.04 t/ha, 18.63 t/ha, and 18.94 t/ha. However, tomato productivity in West Sumatra experienced a decline in 2020, namely 36.60 t/ha, 39.06 t/ha, and 29.79 t/ha, respectively (Badan Pusat Statistik, 2021). The main obstacle is low production tomatoes nationally is a limitation cultivation technology owned by farmers and lack of technological information, such as branch pruning, fruit thinning, cultivation techniques, down to fertilization balanced, uncertain climate. and the low level of fertility of the land to be planted tomato plants (Surtiningsing, 1991; Kaya, 2020). Apart from the problem cultivation technology, plant pest problems such as nematode *Meloidogyne* spp. attacks also play an important role in declining tomato production in Indonesia.

Nematodes *Meloidogyne* spp. is the most important phytonematode in the world, especially in agriculture in tropical areas. The host plant *Meloidogyne* spp. is very broad, including horticultural crops, secondary crops, plantations, and weeds (Dropkin, 1991). *Meloidogyne* spp. have the highest level of penetration and fecundity in tomato plants compared to several plants belonging to the Solanaceae family, such as eggplant, large chilies, and cayenne peppers (Wulandari et al. 2019). Affected plants by *Meloidogyne* spp. usually show abnormal symptoms such as stunting and tend to wilt on hot days, while the roots experience swelling. Attacks on tomato plants mainly occur on soil with a rough or sandy texture; besides weakening the plants, nematodes can also cause yield loss (Sastrahidayat, 1990). Plants that are attacked are characterized by the formation of knots or galls in the root system; the leaves experience chlorosis; the plants are stunted; the leaves wilt and fall off a lot; the roots are fewer; if the plant is attacked heavily or severely, the plant will die (Taylor and Sasser, 1978). The level of root damage to tomato plants caused by the nematode *Meloidogyne* spp. in cycle I was 40%, and in cycle II it reached 68.3% (Khotimah et al., 2020). In high populations it can cause yield losses of 25-50% (Rahayu dan Mukidjo, 1977).

In general, control of plant parasitic nematodes is still carried out using pesticides in the form of insecticides, which can also be used as nematicides.

2. Materials and Methods

Preparation of *Beauveria bassiana* and *Trichoderma asperellum*

The endophytic fungus *Beauveria bassiana* and *Trichoderma asperellum* used were from the collection of the

Continuous use of chemicals to control nematodes can cause environmental pollution, resurgence due to the death of natural enemies, and nematode resistance to chemicals (Winarto et al., 2018). Biological control of nematodes *Meloidogyne* spp. using endophytic fungi can be an environmentally friendly alternative control (Purwanti et al., 2018). Winarto's research results showed that there were 7 types of fungi that could be used to control *Meloidogyne* spp., namely *Paecilomyces* sp., *Penicillium* sp., *Aspergillus* sp., *Fusarium* sp., *Gliocladium* sp., *Chaetomium* sp., and *Trichoderma* sp.

Trichoderma spp. is an antagonistic fungus and is the biological agent most widely used to control various plant pathogens, including fungi, bacteria, and nematodes, and can even control weeds (Muslim, 2019). Antagonistic mechanisms of *Trichoderma* spp. are antibiosis, competition, and mycoparasites (Haran et al., 1996). In vitro, the fungus *Trichoderma harzianum* has the highest nematicidal activity against the nematode *Meloidogyne incognita* when compared with the fungi *Fusarium* sp., *Aspergillus* sp. and *Penicillium* sp. (Devi and Bora, 2018). In the research of Javeed et al. (2016) isolates of *Trichoderma* spp. The most effective method for controlling the *Meloidogyne javanica* nematode on tomato plants is *Trichoderma harzianum*. The one that most commonly parasitizes *Meloidogyne javanica* nematode eggs is *Trichoderma asperellum* with a reduced hatching rate of 82.6 – 88% (Hemeda et al., 2019). *Trichoderma asperellum* strain TT34 and *Trichoderma harzianum* strain T22 can induce tomato resistance to nematode attacks (Pocurull et al., 2020).

Apart from *Trichoderma*, an antagonistic fungus that is reported to be able to control plant pathogens is *Beauveria bassiana*. The fungus *B. bassiana*, which has been reported to control insect pests, also has the ability to control plant pathogens (Gothandapani et al., 2014). The research results of Sanivada and Challa (2014) showed that the fungus *B. bassiana* has antagonistic effects against *Colletotrichum falcatum*, which causes red root disease in sugar cane plants, and *Alternaria porri* in shallots (Gothandapani et al., 2014). The research results of Sun et al. (2006) showed that the *B. bassiana* fungus could infect *M. hapla* eggs, so that the percentage of eggs hatching was low, at only 36.6%. Agustina et al. (2021) reported that *B. bassiana* was able to inhibit the growth of the fungus *Colletotrichum capsici* with an inhibitory percentage of 14.23–15.75%. *B. bassiana* was also able to inhibit colony growth, the number of conidia, and conidial germination of the fungus *C. capsici*. Information on *B. bassiana* ability to control *Meloidogyne* spp has not been widely reported. The aim of the research was to determine the ability of the endophytic fungi *T. asperellum* and *B. bassiana* as nematicides to control root knot nematodes *Meloidogyne* spp. on tomatoes.

Biological Control Laboratory, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Andalas. There were two isolates of *B. bassiana* used, namely TD312 and PB211, and two *T. asperellum* isolates, namely SD324 and S2D11.

The fungus was propagated on SDAY media and incubated for 3 weeks. The growth of colonies of *B. bassiana* and *T. asperellum* can be seen in Figure 1.

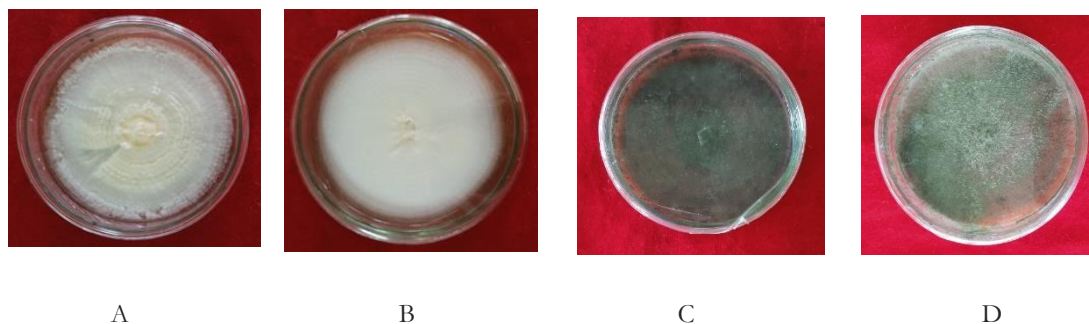


Figure 1. Colony growth of *B. bassiana* and *T. asperellum* on SDAY medium, A (*B. bassiana* TD312), B (*B. bassiana* PB211), C (*T. asperellum* SD324), D (*T. asperellum* S2D11)

Propagation of *B. bassiana* and *T. asperellum* on Rice Media

The propagation of *B. bassiana* and *T. asperellum* using rice is done by washing the rice and then soaking it in a container filled with hot water for 15 minutes, after

which it is placed in a 1 kg heat-resistant plastic bag weighing as much as 100 grams. Next, sterilize using an autoclave for 45 minutes. After cooling, insert pieces of pure culture isolates of *B. bassiana* or *T. asperellum* with a size of $\pm 1 \times 1$ cm into a plastic bag and tie. Cultures were incubated for 21 days at room temperature.



Figure 2. Propagation of *B. bassiana* (A) and *T. asperellum* (B) on rice

Preparation of planting media

The planting medium used in this research was a mixture of sterilized soil and compost with a volume ratio of 3:1. Sterilization is carried out using the tyndalization technique, namely that the planting medium is packaged in heat-resistant plastic measuring 5 kg and placed in a sterile container in the form of a steamer at a temperature of 100 oC for 60 minutes. Then leave the planting medium for 24 hours, then put it in a polybag with a diameter of 30 cm.

Sowing tomato seeds and planting

The tomato seeds used are Warani variety tomato seeds which are known to be susceptible to root swelling nematodes. Seeds are sown in pottrays containing sterile planting medium for 21 days, then transferred to polybags. Seedlings transferred to polybags had good and uniform growth.

Preparation and multiplication of nematode inoculum

The nematode inoculum was taken from a tomato field in Alahan Panjang with symptoms on the tomato plants, namely stunted plants and swelling on the roots. Roots with symptoms of root swelling were taken to the laboratory and then observed for egg clusters. Groups of eggs on the swollen surface were removed and collected in a petri dish filled with water.

Nematode inoculum propagation in wirehouses was carried out by inoculating 5 groups of eggs per 21 day old tomato plant for 10 tomato plants. Then wait until the age of 45 days, the tomato plants are removed and the roots are cleaned with water. Groups of eggs contained in root swellings were collected in petri dishes. To obtain nematode eggs, a suspension of eggs is made from a group of eggs by dripping 2-3 drops of 0.5% NaOCl solution into a petri dish. Then the

number of eggs is counted under a microscope using a hand tally counter.

Application of endophytic fungus and nematode infestation

The fungi *B. bassiana* and *T. asperellum* which have been propagated on rice are mixed with 9 g/kg of soil in the planting medium. The fungus culture was mixed to a depth of 15 cm from the soil surface in a polybag and incubated for 7 days. Then one tomato seedling is planted per polybag. Nematode infestation using ± 1000 eggs per polybag was carried out one week after planting the tomato seeds.

Observation

1. Development of root galls (Meloidogyne spp.)

The development of root galls nematodes (*Meloidogyne* spp.) was carried out 45 days after planting (DAT) with the parameters of observing the

3. Results and Discussion

Nematode Development

Observation results showed that all treatments of the endophytic fungi *Beauveria bassiana* and *Trichoderma*

number of root galls, number of egg groups, number of eggs per egg group and number of nematodes in soil samples.

2. Observation of Plant Growth

a. Plant height

Plant height is calculated from the base of the stem to the growing point of the main stem. Measurements were carried out once a week after the plants were moved into polybags until the plants were 45 days old.

b. Number of leaves

Observation of the number of leaves on tomato plants was carried out by counting the number of leaves that appeared on the plants starting on the 7th day after application of the treatment.

c. First Flowers Appear and production

Observations of the appearance of the first flower are carried out every day until the first flower appears and blooms completely. Tomatoes can be harvested after they are physiologically ripe or 70 days after planting and weighed.

asperellum could suppress the development of *Meloidogyne* spp based on the number of galls, number of egg masses, number of eggs per egg masses and nematode population in the soil. The results of the real difference analysis according to LSD 5% are shown in Table 1.

Table 1. Effects of Endophytic fungi on Nematode Development Average number of galls, egg groups, number of eggs and nematodes in the soil in each treatment

Treatment	Galls	Egg masses	Number of eggs per eggs mass	Nematodes in soil
A	9.25 a	2.50 a	79.50 a	5.25 a
B	14.25 a	3.25 ab	117.50 ab	14.50 b
C	32.25 b	8.25 b	151.50 b	19.25 b
D	34.75 b	8.00 b	131.50 b	15.25 b
E	115.00 c	29.00 c	256.50 c	27.25 c

Information: A (*Beauveria bassiana* isolate TD312), B (*Beauveria bassiana* isolate PB211), C (*Trichoderma asperellum* isolate SD324), D (*Trichoderma asperellum* isolate S2D11), and E (control)

In table 1, it can be seen that the nematode population and number of galls was higher in the control and was significantly different compared to plants treated with endophytic fungi. The application of the endophytic fungus *B. bassiana* is better able to reduce the number of galls, egg groups, number of eggs and nematodes in the soil compared to the fungus *T. asperellum*. The *Beauveria bassiana* fungus can suppress the development of *Meloidogyne* spp because it produces enzymes including chitinase which can damage egg shells thereby affecting the hatching of *Meloidogyne* spp eggs. Apart from that, the *Beauveria bassiana* also produces toxins which can disrupt the nervous system and also kill the larvae of *Meloidogyne* spp., by destroying them. eggs and also the death of larvae will influence the formation of galls. Sun et al. (2006) reported that the *B. bassiana* could infect *M. hapla* eggs so that the percentage of eggs hatching was low at only 36.6%. The mechanism

of the fungus *B. bassiana* in controlling nematodes can be parasitism. According to Bayu et al. (2021) the enzymes produced by *Beauveria bassiana* are chitinase, protease, amylase and lipase, while the toxins produced include beauvericin, bassianin, bassiacridin, bassianolide, cycloporin, oosporein and tenellin. Karaborklu et al. (2022) stated that the root swelling index of *Meloidogyne incognita* in tomato plants decreased from 8.0 in the control to 3.2 in the application of *Beauveria bassiana*. The number of stage II larvae in the soil decreased from 2240 in the control to 508 in the *Beauveria bassiana* treatment. The decrease in the root swelling index and the decrease in the number of larvae in the soil is caused by the presence of secondary metabolites from *Beauveria bassiana* which can damage the egg shells so that the eggs do not hatch and also result in the death of larvae that have hatched from the eggs so that root swelling does not form.

Apart from being able to control pathogens directly, the endophytic *B. bassiana* fungus is also reported to be able to control pathogens indirectly through the mechanism of inducing plant resistance. Induced plant resistance is resistance that develops after plants are inoculated with biotic agents, chemical compounds, and physical treatments. Induced resistance can develop if host cells are able to carry out transcription and produce enzymes or proteins that activate genes responsible for plant defense mechanisms. increased activity of peroxidase, phenylalanine aminia lyase, phytoalexin, lignification, and proteinase inhibiting enzymes was found in several plants that experienced increased induced resistance (Agrios, 1996). Soaking treatment of tomato seeds with *B. bassiana* isolates has been reported to increase the activity of systemic resistance-inducing enzymes such as peroxidase (POX) and phenylalanine ammonia-lyase (PAL) as well as phenolic compounds in tomato plants, thereby suppressing damping-off disease caused by *R. solani* (Azadi et al., 2016). The same thing is thought to have happened to tomato plants that were applied with *Beauveria bassiana* to control *Meloidogyne* spp.

The fungus *Trichoderma asperellum* is also able to suppress the development of *Meloidogyne* spp. This is because the secondary metabolites produced are able to suppress the development of nematodes, both the development and hatching of eggs and the development of *Meloidogyne* larvae. Sibero et al. (2019) stated that secondary metabolites produced by *Trichoderma asperellum* include peptaibols, alkaloids, phenol hydroquinone, flavonoids and saponins. Khan et al.

(2020) stated that the fungus *Trichoderma asperellum* produces secondary metabolites that are toxic to nematodes. These metabolite compounds include volatile compounds which are a mixture of polyketides, butenolactones and terpenoids and other compounds in the form of peptides and enzymes. According to Al-Hazmi and Tariqjaveed (2016), the *Trichoderma* fungus can colonize roots, causing changes in physiology and metabolism and producing secondary metabolite compounds due to the elicitor compounds it produces.

According to Kappel et al. (2020) in general, *Trichoderma* fungi produce secondary metabolites that are antagonistic to nematodes by inhibiting the hatching of nematode eggs. These compounds are tricomycin, gliotoxin, viridin, antibacterial peptide, β -1, 3-glucanase, chitinase, polypeptide, polyketone, butyrolactone, sesquiterpene heptadecarboxylic acid, terpene, and some volatile substances (hydrocarbons, alcohols, furans). aldehydes, alkanes, olefins, esters, aromatic compounds, heterocyclic compounds, and various terpenoids. Saharan et al. (2023) stated that *Trichoderma asperellum* in laboratory tests showed activity as a nematicide against *Meloidogyne incognita* by suppressing egg hatching by 96.6% and larval death by 90.3%.

Growth and Yield of Tomato Plants

Observation results show that treatment of the antagonistic fungi *Beauveria bassiana* and *Trichoderma asperellum* can increase the growth and yield of tomato plants (Table 2)

Table 2. Effects of Endophytic fungi on Plant height, number of leaves, flowering age and yield for each treatment

Treatment	Plant height	Number of Leaves	Flowering Age	Production
E	75.25 a	14.75 a	46.75 a	94.25 a
B	84.00 b	16.50 b	43.00 b	126.75 ab
A	86.50 b	16.75 b	39.25 c	218.75 c
C	89.00 b	18.50 c	41.25 bc	157.75 b
D	89.00 b	18.75 c	39 c	216.75 c

Information: A (*Beauveria bassiana* isolate TD312), B (*Beauveria bassiana* isolate PB211), C (*Trichoderma asperellum* isolate SD324), D (*Trichoderma asperellum* isolate S2D11), and E (control)

Treatment with *Beauveria bassiana* and *Trichoderma asperellum* can increase plant height, number of leaves and yield of tomato plants. The ability of the fungus *B. bassiana* to increase plant growth has been reported by several studies. Liu et al. (2022) stated that *Beauveria bassiana* is an endophytic fungus that can colonize roots and can increase plant growth, increase the length and wet weight of roots, increase stem weight. Trizelia et al. (2020) reported that the *B. bassiana* fungus applied to chili plants by soaking the seeds could increase the growth of chili seedlings. The increase in plant growth is caused by this fungus being able to produce the

growth hormone in the form of Indole Acetic Acid (IAA) as reported by Yusniwati et al (2023).

The fungus *Trichoderma* spp. can produce compounds that can increase plant growth, especially plant height, number of leaves so that yields increase. According to Lombardi et al. (2020), *Trichoderma* produces compounds that promote growth such as indole acetic acid which can encourage plant root growth because it can help absorb nutrients in the soil, increase photosynthesis efficiency, increase plant height, stem diameter and increase production.

4. Conclusions

The results of the research show that the endophytic fungi *T. asperellum* and *B. bassiana* applied to the soil can suppress the number of galls and eggs masses, eggs per egg mass of *Meloidogyne* spp. *Beauveria bassiana* isolate TD312 is the best isolate for suppressing the development of nematode populations and number of galls in tomato plants. Application of endophytic fungi *Beauveria bassiana* and *Trichoderma asperellum* were also increase the growth and yield of tomato plants. Tomato plant production increased by 25.64-56.91% on plants applied with fungus *B. bassiana* and *T. asperellum*.

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bassiana and *T. asperellum* can be considered as an alternative for combating root-knot nematodes as a substitute for synthetic nematicides

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