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Research Article

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Nematicidal activity of root exudates of sengon plant inoculated with endophytic fungi *Nigrospora* sp. to control of root-knot nematode *Meloidogyne* spp.

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ABSTRACT

Root exudates are substances released by roots and may affect activity of soil organisms in the rhizosphere including of plant parasitic nematodes. The research was aimed to determine the effect of different concentrations of root exudates of sengon plant (Paraserianthes falcataria) that had been inoculated with endophyitic fungi Nigrospora sp. to control root-knot nematode, Meloidogyne spp. The study was conducted using sand block test method. Block I was the area of application of the Meloidogyne-J2. Block II was the area between the application of Meloidogyne-J2 and zone of roots, while Block III was the roots zone of plants. All concentration treatments, except 6.25% of root exudates in Block III had lower population of Meloidogyne-J2 and statistically, significantly different from the untreated control. In Block III, concentration of 100% root exudates treatment suppressed the Meloidogyne-J2 population to the level of 20 % of the population in control.

Key words: root exudates, endophytic fungi, *Nigrospora* sp, *Meloidogyne*-J2

INTRODUCTION

The processes mediated by roots in the rhizosphere such as the secretion of root border cells and root exudates are not yet well understood [1]. In recent years, the field of rhizosphere biology has explored the relative importance of root exudates in mediating interactions with neighbouring plants and microbes [2; 3; 4; 5]. Root exudation is part of the rhizodeposition process, which is a major source of soil organic carbon released by plant roots [6; 7] The quantity and quality of root exudates are determined by plant species, the age of an individual plant and external factors like biotic and abiotic stressors. Root exudation clearly represents a significant carbon cost to the plant [8], with young seedlings typically exuding about 30–40% of their fixed carbon as root exudates [9]. Root exudates contain released ions (i.e. H+), inorganic acids, oxygen and water, but mainly consist of carbon-based compounds [10; 3]. These organic compounds can often be separated into two classes: low-molecular weight compounds, which include amino acids, organic acids, sugars, phenolics and an array of secondary metabolites, and high-molecular weight compounds like mucilage and proteins. Root exudates are substances released by roots and may affect activity of soil organisms in the rhizosphere included plant parasitic nematodes. Carbohydrates, amino acids, Flavonols, Lignins, Coumarins, Aurones, Glucosinolates, Anthocyanins, Indole compounds, Fatty acids, Sterols, Allomones, Proteins and enzymes have been identified in the exudates of a wide variety of plants.

Plant parasitic nematodes are responsible for over $ 100 billion dollars in economic losses worldwide to a variety of crops. Root-knot nematodes are the most economically important group of plant parasitic nematodes worldwide, reducing both yield and crop quality [11; 12]. Infected plants show reduced growth, swollen roots which develop into the typical root-knot galls, are two, or three times larger in diameter as healthy root. Root-knot nematodes are very difficult to control because they are polyphagous, where its over 2000 plants species is a highly specialized and

complex feeding relationship with their host [13]. The life cycle is almost completely confined inside the host plant and high reproductive capacity. Although chemical control is still a common method for reducing nematode population, there is a considerable public pressure to limit or even ban the use of nematicides. Many nematicides are highly toxic and sometimes very mobile in the soil because of their solubility in water. Concern over these chemicals has led to an increased interest in biological control in order to achieve more environmentally friendly methods of reducing nematode damage. The objective of this investigation was to know the effect of roots exudates from fungal endophytes *Nigrospora* sp. to control of root-knot nematode *Meloidogyne* spp. on sengon plant (*Paraserianthes falcataria*).

EXPERIMENTAL SECTION

Source of Endophytic Fungi *Nigrospora* Sp.

Endophytic fungi *Nigrospora* sp. was originally isolated from cortical tissue of surface sterilized root of sengon plant *Paraserianthes falcataria*

Source of *Meloidogyne* spp.

The root-knot nematode *Meloidogyne* spp. was originally isolated from an infested field on tomato plant in distric Barombong, south Sulawesi, Indonesia. The extraction to obtained *Meloidogyne*-J2 by using the modified extraction technique of Hooper *et al* [14]. Roots were washed free from soil under tap water, cut into 1 cm pieces and macerated in a warring blender at high speed for 20 seconds and collected in a glass bottle. Sodium hypochloride (NaOCl) was added to obtain a final concentration of 1.5% active Chlorine and the bottle was shaken vigorously for 3 min. The suspension was then thoroughly washed with tap water through a sieve combination 250, 100, 45 and 25 µm mesh to remove the NaOCl. Eggs were collected on the 25 µm sieve and then transferred to a glass bottle. The egg suspension was supplied with oxygen from an aquarium pump over 10 days to induce juvenile hatching. To separate active *Meloidogyne*-J2 from unhatched eggs or dead *Meloidogyne*-J2, a modified Baermann technique over 24 hours was used. The collected active *Meloidogyne*-J2 were adjusted to 1000 *Meloidogyne*-J2 5 ml-1 and used immediately as inoculums.

Preparation of Root Exudates of Endophytic Fungi *Nigrospora* sp.

Preparation of root exudates implemented by petri dishes method. Thirty days aged of sengon plants that have been inoculated with endophytic fungi *Nigrospora* sp. were removed carefully so as not to damage the root structure. The soil particles were removed with sterile water, and then transferred to a petri dishes containing 10 ml of sterile, distilled water (5 thirty days aged of sengon plants per dish). After 5 or more days in the dark at 25^0 C, the thirty days aged of sengon plants and liquid are transferred to asterile beaker, the thirty days aged of sengon plants are rinsed with sterile, and the rinse liquid is combined with the rest of the exudates. The material is dried by evaporation under vacuum at 40^0 C, dissolved in a small volume of water, and then passed through a sellulose membrane filter into a weighed sterile tube. The sterile exudates is redried under vacuum, and the dry weight is determined. Sterile water is then added to make a 10% (w/v) solution.

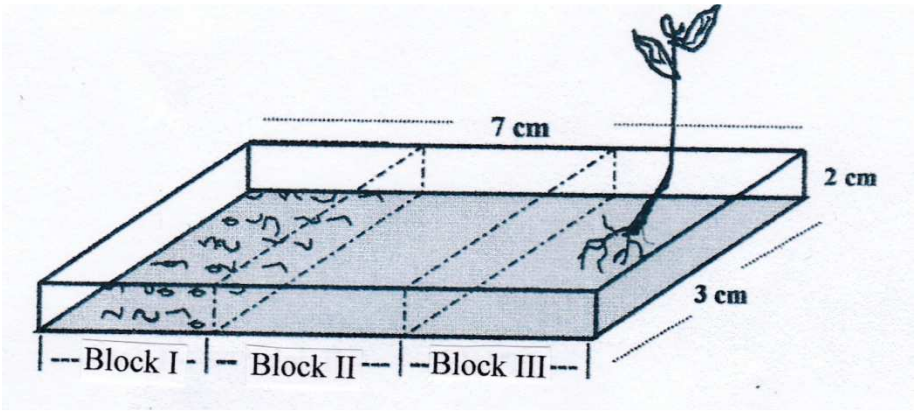

Figure 1. sand Block test Method

Block I : Zone of aplication of root-knot nematode *Meloidogyne* spp.
Block II : Zone between aplication of root-knot Nematode and root zone
Block III : Zone of Roots

Investigation of Root Exudates of Sengon Plant Inoculated With Endophytic Fungi *Nigrospora* sp. In Different Concentration against Root-Knot Nematode *Meloidogyne* spp.

The effects of root exudates on fungal endophyte *Nigrospora* sp. on sengon plant was determined by using of "**Sand Block Test Method**". Sifted and moistened sterile sand was placed into a sand block (7 x 3 cm x 2 cm). In Block I the exudates extract was applied 2.3 cm from 1500 individuals of *Meloidogyne*-J2. Block III is 6 cm from the tip of seedlings planted sengon previously been soaked roots in accordance with their respective treatment for 30 minutes (Figure 1).

RESULTS AND DISCUSSION

In the present study, it was confirmed that the treatment of AS2 (100% root exudates) showed that the number of Meloidogyne-J2 in block III was eight times less than the treatment of AS0 (sterile water control) and significantly different. Similarly, that the treatment of AS3 (50% root exudates) and AS4 (25% root exudates) showed significantly statristically different. It is hypothesized that discrete compounds from crown daisy root exudate may play important roles in regulating nematode behaviour, resulting in a decrease in the damage caused by nematodes to the host in a tomato–crown daisy intercropping system. A number of compounds were identified in the root exudate of tomato or crown daisy; many bioactive compounds may be determinants of alleviating nematode damage. However, only the highly abundant compound (lauric acid) which existed in the root exudate of crown daisy was screened for, relying on the mass spectral database. It is also likely that other compounds in crown daisy root exudate play important roles and, therefore, further studies are required. Although the functions of most root exudates have not been confirmed, an abundance of compounds has been detected in the root exudate [15; 16; 17]. Many crops naturally release nematotoxic compounds into the environment either from their roots or directly from plant tissue to suppress RKNs [18; 19]. It has been demonstrated that the phototoxin α-therthienyl, which has been extracted from asteraceae species, is a major nematicidal compound. This compound may be released into the environment to suppress nematode damage [20]. Lauric acid has been identified as a novel bioactive and high-abundance compound in root exudates of the family asteraceae. However, lauric acid was assayed in a hydroponic culture, from which environmental factors were absent, and so the accumulated lauric acid content in natural intercropping practice is unclear.

Allelochemical in root exudates have been shown to process antimicrobial and antinematicidal activities. Kobayashi *et al* [21] reported that the antimicrobial activity of wheat root exudates is due to presence phenolic acids, sugars, amino acids, and possibly others compounds. Lee and Scagel [22] reported that chicoric acid indicated helps a plant protect itself from insect and infection from viruses, bacteria, fungi and nematode, and that it aids in wound healing in plants after mechanical damage. Beyond the better understood roles it has in seed germination, additional research is needed to better understand why plants produce chicoric acid and to corroborate theories that phenolic acids defend against microbial and herbivore attack by acting as deterrents, toxins, or signaling molecules.

Table 1. Average Number of *Meloidogyne*-J2 7 days after application of Root Exudates of Endophytic Fungi *Nigrospora* sp.

Treatment	Average Number of Larvae Instar II *Meloidogyne* spp.		
	Block I	Block II	Block III
AS0	255 c	363	454 c
AS1	236 c	273	509 c
AS2	636 a	345	91 a
AS3	509 a	345	218 b
AS4	454 b	327	218 b
AS5	146 c	218	618 d
AS6	109 c	363	509 c

Interesting results were found in treatment of AS5 (12.5% Root exudates), which the number of *Meloidogyne*-J2 nematode in block III more than the control AS0. All treatments in block II are not significantly different in the number of nematodes.endophyte) and AS6 (6.25% Root exudates) the number of nematode in block III more than the AS0. This phenomenon illustrates that the administration of the chemicals (exudates) below the recommended dose can induce higher patogenicity of parasitic nematodes. Similar results has been reported by Nur Amin (1994) that more nematode *Radopholus similis* infected the roots of banana plants applied with culture filtrate of endophytic fungus *Fusarium oxysporum* A1 at a concentration of 6.25% then the untreated control. In according to Dong *et al* [23], Low concentrations of lauric acid (0.5–2.0 mM) attract *M. incognita* and consequently cause death, while high concentrations (4.0 mM) repel *M. incognita*. This study elucidates how lauric acid in crown daisy root exudate regulates nematode chemotaxis and disrupts *Mi-flp-18* expression to alleviate nematode damage, and presents a general methodology for studying signalling systems affected by plant root exudates in the rhizosphere. This could lead to the development of economical and feasible strategies for controlling plant-parasitic nematodes, and provide an alternative to the use of pesticides in farming systems.

CONCLUSION

It can be concluded that, root exudates of endophytic fungi *Nigrospora* sp. have nematicidal activity by concentrations of 100, 50 and 25 % against root-knot nematode *Meloidogyne* spp.

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