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# The Effect of Water Extract of Salam Koja Leaf (*Murraya koenigii* (L.) spreng) Against Root-Knot Nematode (*Meloidogyne* spp.) in Tomato Plants

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**ABSTRACT**

Root-knot nematodes (*Meloidogyne* spp.) is one of the pathogens that attack tomato plants so that it can reduce tomato production. One alternative to control *Meloidogyne* spp. environmentally friendly is to use botanical nematicides. Salam Koja (*Murraya koenigii* (L.) Spreng) has potential as a botanical nematicide because its leaves contain secondary metabolites which are antihelmintic. The purpose of this study was to determine the effect of water extract of *M. koenigii* leaves and to obtain the best concentration in suppressing the attack of *Meloidogyne* spp. on tomato plants. This research was conducted at the Greenhouse, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Padjadjaran, Jatinangor. The study used an experimental method with a randomized block design consisting of 6 treatments and 4 replications. The treatments included control (without water extract of *M. koenigii* leaves), concentrations of water extract of *M. koenigii* leaves 6%, 9%, 12%, 15%, and carbofuran 2 g/plant. The results showed that the water extract of *M. koenigii* leaves had an effect on suppressing root-knot nematodes (*Meloidogyne* spp.) on tomato plants. The water extract of *M. koenigii* leaves at the highest concentration of 15% is effective in suppression of the number of galls in the roots (38.57%) and suppression of the number of juvenile 2 (J2) *Meloidogyne* spp. in 100 ml of soil (81.03%). *Murraya koenogii* leaf water extract can be used to control *Meloidogyne* spp. in tomato plants.

Keywords: antihelmintic, botanical nematicides, leaf water extract, plant parasitic nematode

**Pengaruh Ekstrak Air Daun Salam Koja (*Murraya koenigii* (L.) spreng) terhadap Nematoda Bengkak Akar (*Meloidogyne* spp.) pada Tanaman Tomat**

**ABSTRAK**

Nematoda bengkak akar (*Meloidogyne* spp.) merupakan salah satu OPT yang sering menyerang tanaman tomat sehingga dapat menghambat produksi tanaman tomat. Salah satu alternatif pengendalian *Meloidogyne* spp. yang ramah lingkungan adalah dengan menggunakan nematisida nabati. Salam koja memiliki potensi sebagai nematisida nabati karena pada bagian daunnya mengandung senyawa metabolit sekunder yang bersifat antihelmintik. Percobaan ini bertujuan untuk mengetahui pengaruh ekstrak air daun salam koja dan mendapatkan konsentrasi terbaik dalam menekan serangan *Meloidogyne* spp. pada tanaman tomat. Percobaan ini dilakukan di Rumah Kaca, Departemen Hama dan Penyakit Tumbuhan, Fakultas Pertanian, Universitas Padjadjaran. Percobaan ini menggunakan Rancangan Acak Kelompok terdiri dari 6 perlakuan dan 4 ulangan. Konsentrasi ekstrak air daun salam koja yang diuji adalah kontrol, 6%, 9%, 12%, 15% dan karbofuran 2 g/tanaman. Hasil percobaan menunjukkan bahwa aplikasi ekstrak air daun salam koja dapat menekan serangan nematoda bengkak akar (*Meloidogyne* spp.) pada tanaman tomat di rumah kaca. Ekstrak air daun salam koja pada konsentrasi paling tinggi 15 % efektif menekan jumlah *gall* pada akar tanaman tomat sebesar 38,57% dan menekan jumlah juvenile II *Meloidogyne* spp. dalam 100 mL tanah sebesar 81,03%. Ekstrak air daun salam koja dapat digunakan untuk pengendalian *Meloidogyne* spp. pada tanaman tomat.

Kata Kunci: antihelmintik, ekstrak air daun, nematisida botani, nematoda parasit tanaman

## INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most widely cultivated horticultural crops in Indonesia. Tomato production in West Java in 2017 (295,321 tons), 2018 (268,448 tons), and 2019 (284,948 tons) (Ministry of Agriculture, 2019). Tomato production in West Java tends to be unstable because it is influenced by several factors, one of which is plant parasitic nematode which can inhibit plant growth and cause a decrease in yield. Plant parasitic nematode that often attacks tomato plants is root-knot nematodes (*Meloidogyne* spp.).

*Meloidogyne* spp. is a plant parasitic nematode that can attack nearly 2,000 plant species including the Solanaceae family (Agrios, 2005), and can cause yield losses of 25% to 50% (Taylor & Sasser, 1978). *Meloidogyne* spp. attack symptoms are yellowing leaves, stunted plant growth and galls on plant roots (Khan *et al.*, 2017).

The use of synthetic nematicides to control *Meloidogyne* spp. can cause negative impacts such as water and soil pollution, cause resistance and residues on fruit surfaces that are dangerous if consumed by humans or animals (Kardinan, 2001). Therefore we need an alternative control that is environmentally friendly, safe for humans and effective in controlling *Meloidogyne* spp., namely by using botanical nematicides. Botanical nematicides do not leave residues on either plants or the environment because they are easily degraded (Khater, 2012).

Salam koja leaf (*Murraya koenigii* (L.) Spreng) contains carbazole alkaloids, saponins, flavonoids and tannins which can inhibit the development of nematodes or have antihelmintic properties (Handral *et al*., 2012). Based on the statement of Chitwood (2002), alkaloid group compounds are nematotoxic because these compounds can inhibit the movement of juveniles, inhibit egg hatching and cause death in *Meloidogyne* spp. Usman & Siddiqui (2013), reported that *M. koenigii* extract can be used as a bare-root dip treatment which can significantly reduce the nematode *Rotylenchulus reniformis* population at an extract concentration of 10% and stimulate plant growth. Afzal *et al*. (2013), also reported that leaf extract of *M. koenigii* has significant antihelmintic activity, ethanol extract of leaf of *M. koenigii* at a concentration of 500 mg/ml can cause paralysis and death of *Pheretima posthuma* worms, respectively 11 seconds and 17.5 seconds. Wardani *et al*. (2015) stated that *M. koenigii* leaf extract at a concentration of 12% could cause nematode egg mortality of 71.38% within 10 days and second juvenile nematode (J2) mortality up to 100% within 21 hours. Based on this, it is necessary to conduct research to determine the effectiveness of *M. koenigii* leaves in suppressing root-knot nematodes (*Meloidogyne* spp.) on tomato plants. The purpose of this study was to determine the effect of water extract of *M. koenigii* leaves and to obtain the best concentration in suppressing the attack of *Meloidogyne* spp. on tomato plants.

## MATERIALS AND METHODS

The research was conducted at the Phytopathology Laboratory, Plant Nematology Laboratory Division and Greenhouses, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Padjadjaran, Jatinangor from February to August 2022.

The tools used in this experiment were an autoclave, blender, pipette, beaker glass, hand counter, Baermann funnel, microscope, analytical balance, plastic container, poly bag, nematode sieve sizes of 750 µm, 50 µm and 35 µm , petri dishes, cloth, knife, ruler and camera. The materials used were tomato seeds of the Intan variety, distilled water, 0.5% sodium hypochlorite solution, fresh *M. koenigii* leaves, water, pasteurized growing media and a source of inoculum nematodes *Meloidogyne* spp.

### Research methods

This study used an experimental method with a randomized block design (RBD) consisting of 6 treatments and 4 replications. Treatment as follows:

A. Control (without water extract of *M. koenigii* leaves)
B. Concentration of water extract of *M. koenigii* leaves 6%
C. Concentration of water extract of *M. koenigii* leaves 9%
D. Concentration of water extract of *M. koenigii* leaves 12%
E. Concentration of water extract of *M. koenigii* leaves 15%
F. Carbofuran 2 g/plant

Each treatment was inoculated with 2,000 second juvenile (J2) *Meloidogyne* spp. Application of 50 mL of *M. koenigii* leaf water extract per treatment polybag. Data were analyzed by analysis of variance (ANOVA) using SPSS version 25.0. To differentiate between the treatment averages, a follow-up test was carried out with Duncan's Multiple Range Test at 5% significance level.

### Planting media, Seeding, and Planting Tomato Plants

The planting medium consists of a mixture of soil, husk charcoal and pasteurized compost with a ratio of 1:1:1. Tomato seeds were sown in seed trays for 4 weeks. After the tomato seedlings were 4 weeks old, they were transplanted into treatment pots containing 2 kg of pasteurized soil.

### Preparation of Water Extract of *M. koenigii* Leaves

*M. koenigii* leaves are cleaned using water and then dried. Then the *M. koenigii* leaves were weighed according to the concentration to be tested (w/v). Leaves were weight 6, 9, 12, 15 g respectively. Then each added water to a volume of 100 ml, then blended. The results of the blender are poured into a closed container and left for 24 hours. After 24 hours, the extract is filtered using a cloth, then stored in a place that is not exposed to sunlight. Leaf water extract was applied as much as 50 ml per plot.

### Inoculum preparation of *Meloidogyne* spp.

Inoculum *Meloidogyne* spp. taken from Ciwidey. Root parts of tomato plants infected by *Meloidogyne* spp. washed thoroughly, then the roots are cut into pieces 0.5-1 cm long. Then the roots were

immersed in a 0.5% sodium hypochlorite solution in a beaker glass and stirred for 5 minutes. The solution was filtered using a 750 µm, 50 µm and 35 µm graduated filter. The results of filtering on a 50 µm and 35 µm graduated filter were rinsed with water and accommodated in a beaker glass. Total second juvenile (J2) *Meloidogyne* spp. counted using a microscope and standardized the number of J2 *Meloidogyne* spp. in nematode suspension, by taking 5 samples of nematode suspension each 10 µL, then the average amount of J2 is converted to 1 mL.

**Inoculation of J2 *Meloidogyne* spp. on Tomato Plants**

Inoculation of J2 *Meloidogyne* spp. performed on 4 weeks old tomato plants at the time of transplanting. Inoculation was carried out by pouring the nematode suspension into the holes around the roots of the tomato plants at a hole depth of about 1 cm (Harni *et al*., 2007). The nematodes inoculated into the soil were second juvenile *Meloidoyne* spp. as many as 2,000 heads per polybag.

***M. koenigii* Leaf Water Extract Application**

The application of water extract of *M. koenigii* leaves was carried out one day after the tomato plants were inoculated with *Meloidogyne* spp. *M. koenigii* leaf water extract was sprinkled on each polybag according to the treatment, and the extract was watered once.

**Observation**

Observations were conducted 35 days after nematode inoculation. Observations were conducted on the number of galls on the roots of tomato plants, the amount of second juvenile (J2) *Meloidogyne* spp. in 100 mL of soil, plant height, fresh weight of the shoot plant and fresh weight of the roots.

Number of galls in the roots of tomato plants. Tomato plants are removed carefully, then the roots are cleaned with water. The number of galls on the roots of each plant was counted. Total second juvenil (J2) *Meloidogyne* spp. in 100 mL soil. The soil in the polybag is stirred until homogeneous, then 100 mL of soil is taken. Soil samples were extracted using the Baermann funnel method and incubated for 24 hours. Then the number of J2 *Meloidogyne* spp. in 100 mL of soil was counted using a binocular microscope. Tomato plant height was measured from the base of the stem to the top of the tomato plant. Shoot and root fresh weight of tomato plants. Measurement of the fresh weight of the shoot of the plant and the root part of the plant is done by first removing the plant from the planting medium, then cutting the base of the stem and washing each part thoroughly with water. Then each part was weighed using an analytical balance.

## RESULTS AND DISCUSSION

**Number of Galls in the Roots of Tomato Plants**

The application of *M. koenigii* leaf water extract has the effect of reducing the number of galls on the roots of tomato plants compared to the control (without *M. koenigii* salam water extract) (Table 1). Application of water extract of *M. koenigii* leaves at a concentration of 15% produced the least amount of gall (148.50 gall) with an emphasis on the number of galls of 38.57%, but significantly different from the number of galls in the control, water extract of *M. koenigii* leaves at a concentration of 9% and carbofuran 2 g/plant. The suppression of the number of galls in plant roots is influenced by secondary metabolites in the water extract of *M. koenigii* leaves which are anthelmintic which can inhibit the development and movement of *Meloidogyne* spp.

Table 1. The average number of galls on the roots of tomato plants and the percentage of suppression at 35 DAI (Days After Inoculation)

| Treatment | Average Number of Gall at the root | Percentage of Suppression (%) |
|---|---|---|
| Control (without water extract of *M. koenigii* leaves) | 241.75 ± 67.36 c | 0.00 |
| Concentration of water extract of *M. koenigii* leaves 6% | 189.25 ± 35.17 bc | 21.72 |
| Concentration of water extract of *M. koenigii* leaves 9% | 227.50 ± 23.75 c | 5.89 |
| Concentration of water extract of *M. koenigii* leaves 12% | 169.25 ± 32.07bc | 29.99 |
| Concentration of water extract of *M. koenigii* leaves 15% | 148.50 ± 23.08 b | 38.57 |
| Carbofuran 2 g/plant | 73.25 ± 45.61 a | 69.70 |

The mean value followed by the same lowercase letter is not significantly different according to Duncan's Multiple Range Test at 5% significance level.

*M. koenigii* leaf water extract contains secondary metabolites such as alkaloids, tannins, saponins and flavonoids (Handral *et al*., 2012). According to Gommers (1973), alkaloids, terpenoids, phenolics and tannins act as nematicides which inhibit the development of *Meloidogyne* spp. This is supported by the statement of Adegbite & Adesiyan (2006) that alkaloids, saponins, and flavonoids are combinations that can interfere with nematode hatching, cause paralysis and death of worms because these compounds inhibit brain hormones, edikson hormones and growth hormones (Sinaga, 2009).

Tannin compounds are able to precipitate proteins and react with the larval cuticle cell walls so that they can stop the nematode muscle response to acetylcholine which results in paralysis and death

(Nezriyetti & Novita, 2012). Molan *et al*. (2002), also stated that tannin compounds can interfere with the nematode life cycle and have the potential to inhibit nematode hatching and development so that they cannot infect plant roots. This can affect the formation of galls on plant roots, because the fewer the number of root-knot nematodes, the fewer galls formed.

**Total second Juvenil (J2) *Meloidogyne* spp. in 100 mL Soil**

The application of *M. koenigii* leaf water extract has an effect on reducing the number of J2 *Meloidogyne* spp. in 100 mL of soil compared to the control (without water extract of *M. koenigii* leaves) (Table 2). The application of water extract of *M. koenigii* leaves at a concentration of 15% resulted in the amount of J2 *Meloidogyne* spp. the lowest (5.50 individuals) with the highest suppression of J2 (81.03%), but not significantly different from the amount of J2 in the application of *M. koenigii* leaf water extract at concentrations of 6%, 9%, 12% and carbofuran 2 g/plant. This is because the high concentration used affects the amount of secondary metabolite compounds contained in the extract.

Table 2. Average number of second juvenile (J2) *Meloidogyne* spp. in 100 mL of soil and the percentage of pressure at 35 DAI

| Treatment | Average amount of second juvenil in 100 ml of soil (tail) | Percentage of pressure (%) |
|---|---|---|
| Control (without water extract of *M. koenigii* leaves) | 29.00 ± 5.00 b | 0.00 |
| Concentration of water extract of *M. koenigii* leaves 6 % | 7.25 ± 2.86 a | 75.00 |
| Concentration of water extract of *M. koenigii* leaves 9% | 7.25 ± 7.00 a | 75.00 |
| Concentration of water extract of *M. koenigii* salam koja leaves 12% | 7.75 ± 3.96 a | 73.28 |
| Concentration of water extract of *M. koenigii* leaves 15% | 5.50 ± 3.77 a | 81.03 |
| Carbofuran 2 g/plant | 6.75 ± 1.09 a | 76.72 |

The mean value followed by the same lowercase letter is not significantly different according to Duncan's Multiple Range Test at 5% significance level.

The high percentage of suppression of second juvenille in the soil is due to the secondary metabolites contained in the water extract of *M. koenigii* leaf which is applied to the soil. The more bioactive compounds that are toxic around the soil, the fewer the number of nematodes that can survive (Wardhiany *et al*., 2014). Wardani *et al*. (2015), reported that the application of *M. koenigii* leaf extract at a concentration of 12% had a significant effect on the mortality of second juvenille *Meloidogyne* spp. by 100% within 21 hours in vitro and the percentage of second juvenille mortality can increase with increasing the amount of extract concentration given. Cahyadi (2009), stated that alkaloid and flavonoid compounds can interfere with and inhibit the digestion of nematodes because these compounds are stomach poisons. In addition, alkaloid compounds can affect the nervous system by stopping nerve cell impulses, causing paralysis and death of worms (Hamzah *et al*., 2016).

Flavonoids are chemical compounds found in the water extract of *M. koenigii* leaves. Flavonoids can inhibit breathing or are respiratory poisons because these compounds enter the body of the larvae through the respiratory system so that they will cause paralysis and death in nematodes (Lopez *et al*., 2005). This statement is also supported by Diantari *et al*. (2015), who stated that flavonoid compounds can affect the development and activity of nematodes in the soil.

**Tomato Plant Height**

*M. koenigii* leaf water extract had no effect on tomato plant height (Table 3.). The highest average plant height was found in the application of carbofuran of 2 g/plant (36, 37 cm), followed by the application of 9 % (31,37 cm) and 15% (28.30 cm) of *M. koenigii* water extract. Meanwhile, the control had the lowest plant height of 23.60 cm.

Plant height increases due to the addition of nutrients from the water extract of *M. koenigii* leaves, as well as good distribution of nutrients from the roots to the shoot of the plant so as to support plant growth. This was explained by Subba Rao (1994) in Sunarto *et al*. (2002), that added organic matter indirectly affects plant growth by providing nutrients for plants and improving soil structure and soil aeration so that plants can grow well. Meanwhile, plants infected by *Meloidogyne* spp. function will be disrupted because the results of photosynthesis that accumulate in the root gall are used for the growth and reproduction of *Meloidogyne* spp. (Santo *et al*., 2019). There are other factors that can cause plant height to not differ significantly, such as the presence of pathogens at the start of plant growth (Wardhiany *et al*., 2014).

Table 3. Average height of tomato plants at 35 DAI at several concentrations of water extract of M. koenigii leaves

| Treatment | Average plant height (cm) |
|---|---|
| Control (without water extract of *M. koenigii* leaves) | 23.60 ± 6.95 a |
| Concentration of water extract of *M. koenigii* leaves 6% | 24.37 ± 8.95 a |
| Concentration of water extract of *M. koenigii* leaves 9% | 31.37 ± 0.96 a |
| Concentration of water extract of *M. koenigii* leaves 12% | 23.95 ± 5.89 a |
| Concentration of water extract of *M. koenigii* leaves 15% | 28.30 ± 7.77 a |
| Carbofuran 2 g/plant | 36.37 ± 11.41 a |

The mean value followed by the same lowercase letter is not significantly different according to Duncan's Multiple Range Test at 5% significance level

Plant height increases due to the addition of nutrients from the water extract of *M. koenigii* leaves, as well as good distribution of nutrients from the roots to the shoot of the plant so as to support plant growth. This was explained by Subba Rao (1994) in Sunarto *et al*. (2002), that added organic matter indirectly affects plant growth by providing nutrients for plants and improving soil structure and soil aeration so that plants can grow well. Meanwhile, plants infected by *Meloidogyne* spp. function will be disrupted because the results of photosynthesis that accumulate in the root gall are used for the growth and reproduction of *Meloidogyne* spp. (Santo *et al*., 2019). There are other factors that can cause plant height to not differ significantly, such as the presence of pathogens at the start of plant growth (Wardhiany *et al*., 2014).

**Shoot and Root Fresh Weight of Tomato Plants**

Based on the test results, it was found that the water extract of *M. koenigii* leaves had no effect on the average shoot and root fresh weight of tomato plants after the ANOVA test was carried out, so Duncan's further test was not carried out. In Table 4, it can be seen that the average fresh weight of plant shoot and plant roots in all treatments did not differ much when measured at 35 HSI. The highest average fresh weight of the shoot part of the plant and the root part of the tomato plant was obtained at a concentration of 9% water extract of *M. koenigii* leaf with a weight of 20 g and 7 g, respectively. Meanwhile, the concentration of 15% water extract of *M. koenigii* leaves resulted in an average fresh weight of the shoot and root parts of 17.75 g and 4.5 g respectively compared to other *M. koenigii* leaf extract treatments.

The number and size of galls formed on plant roots can affect the fresh weight of plant shoot. Attack of *Meloidogyne* spp. can cause damage to plant root tissue so that the transportation of water and nutrients from the soil is disrupted. Ayuob (1977) explained that if the transport of water and nutrients by plant roots is disrupted, it will affect the growth of the shoot of the plant directly. The fresh weight of plants is also influenced by the water content contained in the plant cells whose levels can be affected by environmental factors such as temperature and humidity (Sitompul & Guritno, 1995).

Table 4. Average fresh weight of shoot and roots of tomato plants at 35 DAI

| Treatment | Average shoot fresh weigh of plant (g) | Average fresh weight of plant roots (g) |
|---|---|---|
| Control (without water extract of *M. koenigii* leaves) | 17.00 ± 2.55 a | 6.25 ± 1.79 a |
| Concentration of water extract of *M. koenigii* leaves 6% | 17.25 ± 9.73 a | 6.00 ± 3.00 a |
| Concentration of water extract of *M. koenigii* leaves 9 % | 20.00 ± 2.45 a | 7.00 ± 1.58 a |
| Concentration of water extract of *M. koenigii* leaves 12% | 17.50 ± 8.62 a | 5.00 ±1.87 a |
| Concentration of water extract of *M. koenigii* leaves 15% | 17.75 ± 6.53 a | 4.50 ± 2.06 a |
| Carbofuran 2 g/plant | 11.50 ± 4.50 a | 2.50 ±1.11 a |

The mean value followed by the same lowercase letter is not significantly different according to Duncan's Multiple Range Test at 5% significance level.

The fresh weight of plant roots can be caused by the large number of galls formed on the roots due to infection by nematodes *Meloidogyne* spp. The amount of nematodes in the soil can affect the number of galls formed on plant roots and this can affect the fresh weight of plant roots. Nematodes that infect plant roots will suck the cell fluids in the roots as food and secrete enzymes that can trigger cell division so that the roots become swollen (Syahrina, 2022). Root galls that are formed can hinder the transportation of water and nutrients from the roots to the shoot of the plant, especially the leaves, so that the photosynthesis process is disrupted and inhibits plant growth and can reduce plant fresh weight (Sasser & Taylor, 1978).

**CONCLUSIONS**

Based on the experimental results, it can be concluded as follows:

Water extract of *Murraya koenigii* leaves has an effect on suppressing root-knot nematode (*Meloidogyne* spp.) attack on tomato plants. Water extract of *M. koenigii* leaves at the higest concentration of 15% is effective in suppression of the number of galls in the roots (38.57%) and suppression of the number of second juvenile (J2) *Meloidogyne* spp. (81.03 %). *Murraya koenogii* leaf water extract can be used to control *Meloidogyne* spp. in tomato plants.

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