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Nematicidal Activity of Turmeric Extract against Nematodes *Meloidogyne spp.*

Maria Ulfa[1*)], Toto Himawan[2)] and Hagus Tarno[2)]

[1]Plant Pathology Study Program, Faculty of Agriculture, University of Brawijaya
[2]Department of Plant Pest and Diseases, Faculty of Agriculture, University of Brawijaya

Email Address : ulfamahsinmaria@gmail.com

KEYWORDS

Turmeric;
Nematodes;
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Abstract In an effort to reduce the impact of synthetic nematicides, a new formula was developed using plant-based nematicides that are more environmentally friendly, one of the basic ingredients that can be used is turmeric. The purpose of this study was to determine the effectiveness of turmeric extract (*Curcuma domestica*) with various different solvents namely aquades, methanol, and n-hexane at concentration of 5%, 10%, 15% and 20% respectively in inhibiting the activity of *Meloidogyne* spp. The experiment used was a Completely Randomized Design (CRD). Each treatment was repeated 4 times with 4 controls so that there were 52 experimental units. The result of different research data was analyzed using Analysis of Variance (ANOVA) which was then further tested with Honest Significant Difference (HSD) at the 5% level if real, and t-test for in vivo test. Based on the tested extract, it was found that turmeric extract with various solvent (aquades, methanol, and n-hexane) was effective in inhibiting the activity of *Meloidogyne* spp. nematodes outside the roots (hatching eggs and penetration), turmeric extract didn't affect the activity of *Meloidogyne* spp. in the roots (development and reproduction), vegetable nematicides which the most active in suppressing the population and inhibiting the activity of nematodes was turmeric extract using methanol as a solvent at a concentration of 20%.

Introduction

Nematodes come from the Greek language which means thread, elongated like a tube and a coil that can move like a snake (Shah & Mahamood, 2017). Nematodes are cellular organisms. In the soil, nematodes are near the soil surface and around plant roots (rhizosphere) and become organisms that negatively affect plant growth and development (Bernard *et al*., 2017)

Nematodes attack almost all types of plants because nematodes are polyphagous so they are everywhere. These microorganisms are generally in the soil and can survive for a long time. In general, nematode attacks cause damage to roots, by penetrating and sucking root cells so that tissue vessels are disrupted, and translocation of water and nutrients is inhibited (Kafle, 2013). Furthermore, nematode attacks can also affect the process of photosynthesis and transpiration (Shakya & Yadav, 2020) which results in stunted plant growth, wilting, and decreased plant production.

Root-knot nematode (*Meloidogyne* spp.) is one of the main plant parasitic nematodes on several important crops such as Tomato (*Lycopersicum esculentum* Mill), Cabbage (*Brassica oleracea*), Onion (*Allium cepa*), Celery (*Apium graveolens* L) and other crops. Root-knot nematodes (NPA) grow very quickly and have a high compressive power to plant growth (Mukhtar *et al*., 2018). Symptoms of the attack are generally seen on the roots, which are in the form of nodules called root ulcers/swollen roots (Sikder & Vestergård, 2020).

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In Indonesia, NPA attacks cause significant crop yield losses. Yield loss due to infection with *Meloidogyne* spp. varies, depending on crop varieties and environmental conditions, averaging up to 25% of production, meanwhile the economic losses caused by this nematode infection to cultivated plants can reach 14%. It was reported that NPA is a major problem in three provinces (West Java, East Java, and North Sumatra) causing tomato yield losses of around 32%-71%. Based on data from BPS and the Directorate General of Horticulture in 2011 it is known that the yield of tomato plants in West Nusa Tenggara Province from 2007-2011 experienced a decline rate of up to 6.75% per year due to the attack of *Meloidogyne javanica*.

Efforts to control nematodes are generally carried out chemically using synthetic nematicides. Nematicides that are often used to control root-knot nematodes are usually fumigants and non-fumigants (Subedi *et al*., 2020). The problem is that the use of nematicides can have a negative impact on agricultural products and the environment, especially if the use of nematicides is too excessive. Therefore, experts have been trying to find alternative control, namely environmentally friendly control methods such as technical culture, using resistant varieties, biological control with microorganisms and the use of several plant extracts as ingredients for vegetable pesticides (Lu *et al.*, 2020)

However, on the way, biological control is still difficult to do. The problem is that there are often ineffective mass propagation and formulations. Another alternative that allows easier and cheaper application is control with botanical pesticides. Vegetable materials are environmentally friendly because these materials are easily degraded in nature, so they are safe for humans and the environment. In addition, vegetable materials will not cause pest resurgence or other side effects, they can actually save natural enemies (Junaid *et al*., 2013). Plant extracts have the potential to have antifungal activity because they contain secondary metabolites in the form of tannins, flavonoids, terpenoids, and alkaloids. Secondary metabolites are the result of plant metabolism that are not used for growth and are mostly found in old root, stem, and leaf tissues (Sikder & Vestergård, 2020)

The use of botanical pesticides is still very limited, especially by using family findings such as galangal rhizome, turmeric and ginger. In fact, the ability of secondary metabolites contained in this group of plants to inhibit microbial growth is very promising (Permatananda *et al*., 2020). The results showed that the polar extract of turmeric rhizome (*Curcuma domestica*) with concentrations of 40%, 20%, 10%, and 5% could inhibit the growth of *Staphylococcus aureus* and *Pseudomonas* sp. (Sepahpour *et al*., 2018). Galangal rhizome is widely applied to control diseases caused by bacteria such as bacterial wilt and neck rot.

Some of these research reports show that the extract of the curcuma is also effective in controlling pests and diseases, especially those caused by fungi and bacteria. It is likely that a similar effect can also be expected with applications to NPAs. Therefore, a study has been conducted on the effectiveness of several vegetable extracts of the findings to inhibit the activity of the root-knot nematode, *Meloidogyne* spp.

Based on research from it was found that among the three types of plant findings in the form of turmeric, ginger and galangal with a concentration of 7.5%-10% per milliliter turmeric has the highest effect in inhibiting the activity of the nematode *Meloidogyne* spp.

Materials and Methods

Agar Media Preparation

Agar media (2%) was prepared by dissolving 20 grams of agar into 1000 ml of

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distilled water. Then the agar solution was poured into 4 500 ml Erlenmeyer glasses with 250 ml each. After that, all the ingredients were put in an autoclave to be sterilized for 60 minutes. After that it was cooled to 400C, so that it was poured into Petri dishes with a thickness of about 2 mm. About 50 agar plates were prepared, allowed to freeze and ready to use.

Meloidogyne Egg Suspension Preparation

Nematode egg suspension was prepared by extracting the eggs of *Meloidogyne* spp. from infected water henna plant roots. The infected plant roots were cut into pieces of about 2 cm then added 900 ml of water and added with 100 ml of Bayclean (5% NaOCl) so that the solution became 0.5% sodium hypochlorite then blended at high speed for 15 seconds. The mixture was added with 20 ml of Bayclean (5% NaOCl) so that the mixture became a 0.5% sodium hyphochlorite solution and stirred rapidly for 2 minutes (Barker, 1985). The mixture containing eggs was then filtered serially through 60, 100, 200, and 500 mesh sieves so that the eggs were collected on a 500 mesh sieve (Krikpatrick and Sasser, 1983). Next, the 500 mesh filter was rinsed with running water and the eggs were poured into a 50 ml beaker. Eggs are immersed in a solution of sodium hyphochlorite 0, 5% for 2 minutes and then washed with sterile distilled water four times. In this process at the same time identification of nematode species is carried out with butt prints. In *Meloidogyne* butt print, the main characteristic is that there is a lateral line that separates the dorsal and ventral arches. Next, the egg inoculum was prepared by adjusting the density of the suspension to about 65 eggs per 0.1 ml.

Vegetable Extract Preparation

The part of the plant used is the rhizome of each material, namely Galangal, Turmeric, and Ginger. The rhizomes are crushed using a stone crusher. Then, the results of the scour are blended until they are shaped like a fine powder. Powder from each ingredient as much as 100 grams was soaked in 1000 ml of distilled water to make an extract and then stored for 24 hours. Next, the solution is filtered with gauze, and stored in a plastic bottle. For the extract to be given organic solvent ethanol or methanol filtered using a Buchner funnel lined with filter paper, the crude extract is ready for use.

In Vitro Test

The turmeric content was tested first using LC/GC-MS. Then to see the effect of treatment on hatching eggs on the agar surface (in Petri dishes) 0.1 ml of suspension containing 65 eggs was inoculated. Then air-dried in Laminar Air Flow in an open state for several minutes. After the water (from the egg suspension) dries, 0.1 ml of the extract from each treatment is pipetted over the egg collection (the former water container that has dried). There were 3 treatments each repeated four times and four units were prepared as controls, so there were 52 petri dishes. Petri dishes were closed and glued with parafilm and then incubated in the dark at room temperature. The number of hatched eggs was observed every day for 14 days. The number of eggs that hatch is determined.

In Vivo Test

Tomato seedlings aged 15 days were planted in polybags filled with a mixture of soil and sand (2:1). Plants were inoculated with 65-75 eggs per plant by pipetting the larval suspension in 5 holes made as deep as the roots. In each hole, 0.1 ml of a suspension containing about 13-15 eggs was pipetted. Furthermore, the plants were treated with extracts at the time of planting by sprinkling the extracts on the planting media according to the treatment. There were 10 treatments, each of which was repeated 3 times so that there were 30 experimental plants. Plants are

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maintained according to planting standards. After 1 week, the plants were dismantled and the puru formed was observed.

Nematode Mortality Calculation

Percentage of deaths is corrected if there are deaths in the control (provided that the percentage of deaths is 20%).

P = XY/X x 100%

P = Percentage of mortcorrected quality
X = Percent live on contro
Y = Percentage living on treatment

Table 1. Relationship between LD50 and Toxicity Criteria (Bhowmik, 2012).

Category	LD50 (mg/kg)
Super toxic	<-5
Very toxic	5-50
Toxic	50-500
Medium toxic	500-5000

Data analysis

Observational data were analyzed by Analysis of Variance (ANOVA) used Microsoft Excel for in vitro test which was then further tested with Honest Significant Difference (HSD) at the 5% level if real, and t-test for in vivo test.

Results and Discussion

Treatment with vegetable extracts was able to inhibit hatching and egg development (J2) of *Meloidogyne* spp. The percentage of mortality in the treatment using turmeric vegetable extract with various solvents was significantly higher than in the control (Figure 1).

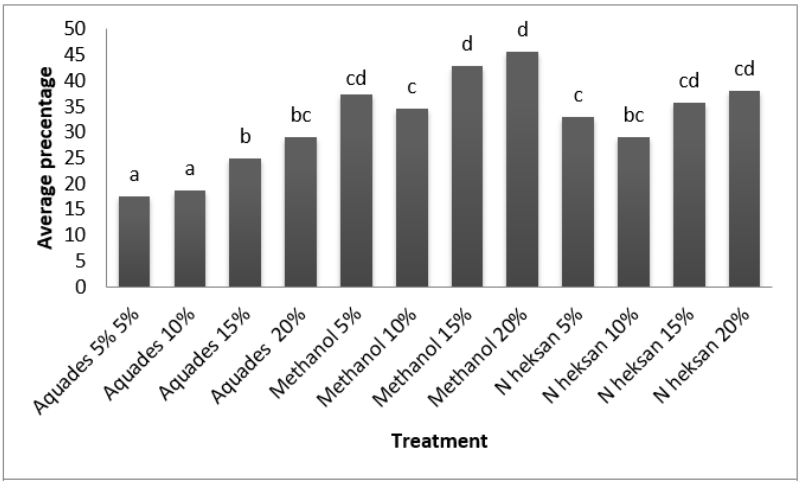

Figure 1. The mean percentage of nematode mortality that was applied to vegetable extracts on 14th day of observation.

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Based on the results of the analysis using GCMS, some of the ingredients identified in turmeric compounds using methanol and n-hexane as solvents, one of which is Zingiberene which is suspected to have a strong influence on the inhibition of nematode activity (Table 2).

Table 2. Results of Analysis of Turmeric Extract with Methanol Solvent

Turmeric content	%
Trans Beta Caryophyllene	2.42
1-Butamine N,N-dimethyl (CAS)	3.18
Benzene	23.55
Zingiberene	4.38
Beta Sesquiphelandrene	5.19
Hexadecanoic Acid	4.88
Octadecenoic Acid/Oleic Acid	35.22
TOTAL	78.82

Table 3. Results of Analysis of Turmeric Extract with N-Hexane Solvent

Turmeric content	%
Trans Caryophyllene	3.00
Benzene	3.42
Zingiberene	5.93
Beta Sesquiphellandrene	6.31
Hexadecanoic Acid	24.66
Palmitic Acid	6.64
Octadecanoic Acid/Oleic Acid	30.48
Benzenedicarboxylic Acid	3.21
TOTAL	83.65

In-vivo test results showed that treatment using turmeric extract with methanol as a solvent was effective in inhibiting the development of *Meloidogyne* both inside and outside the tissue but not significantly different.

Table 4. In Vitro Test by Comparing the Yield of Plants Treated with Extracts That Had the Highest Toxicity with Plants without Nematicide Extract Treatment (Control)

Variable	Treatment (mean ± SD)		Significance
	Control	Methanol 20%	
Number of gall	25.00±10.30	8.75±3.50	*
Diameter of gall	0.22±0.06	0.15±0.07	*
Number off egg sacs	7.00±4.32	2.75±0.96	*

Note : Tested by using t-test, * = non significant, ** = significant

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Turmeric extract with several solvents significantly inhibited egg hatching and penetration of *Meloidogyne* spp into the roots, but had no significant effect on the development and reproduction of *Meloidogyne* in the suppression of the number of hatching eggs and the amount of *Meloidogyne* that penetrated the roots by the treatment of vegetable extracts compared to the control. During these 14 days the number of eggs hatched in the control increased sharply, while in the vegetable extract treatment it remained inhibited until the end of the experiment.

In this study, turmeric extract had a great inhibitory effect on the hatching of the *Meloidogyne* nematode, which was above 50%. Among the vegetable extracts used, the highest inhibitory power was shown by turmeric extract with methanol solvent with a concentration of 20%, followed by 15% methanol and 20% n-hexane. Indonesian spices and herbs contain many anti-microbial compounds. One of them is turmeric (*Curcuma domestica*) which is reported to contain ingredients that can function as anti-microbial compounds. The inhibition of microbial growth is caused by the active compounds contained in turmeric such as essential oils, alkaloids, flavonoids, tannins, curcuminoids and terpenoids (Permatananda, 2020). According to (Liu *et al*., 2020), flavonoid compounds can damage cell walls, causing cell death. In this study, the Zingiberene extract contained in turmeric, which is strongly suspected to be a compound that affects the control of the extract against nematodes, can be seen in both solvents with the results of the test being relatively the same (not significantly different). On the first day to the 9th day, turmeric extract with 20% n-hexane solvent was stronger in controlling nematodes seen from the suppression of the nematode population, then on the 9th to 14th day, the extract with 20% methanol solvent was close to the ability of n-hexane and even exceeded up to 15% which is strongly suspected to be a compound that affects the control of extracts against nematodes can be seen in both solvents with the results of the % area test being relatively the same (not significantly different). In another case, it was also reported that a turmeric solution with a concentration of 5% has shown inhibition of *Staphylococcus aureus* and and *Pseudomonas* sp. The inhibitory effect was stronger at concentrations of 10%, 20% and 40 (Sepahpour *et al*., 2018).

spp. This means that this turmeric extract is effective in inhibiting the activity of *Meloidogyne* spp. in the root. The suppression of *Meloidogyne* activity outside the root can be seen

Inhibition of hatching of Meloidogyne eggs by the extract tested in this study is thought to be through a mechanism similar to that of the other organisms mentioned above. The inhibition of hatching or not hatching of Meloidogyne eggs was caused by the toxic active ingredient of vegetable nematicide (turmeric) which entered the eggs of *Meloidogyne* spp. (Permatananda *et al*., 2020) argues that compounds that can penetrate nematode eggs can affect egg hatching. Therefore, it is very possible that the active ingredients contained in turmeric can penetrate the eggshell of *Meloidogyne* spp thereby inhibiting the enzyme malic dehydrogenase which causes blocking of the intermediate fumarate reductase pathway in metabolizing carbohydrates which further interferes with the formation of energy (ATP) needed for hatching eggs (Sepahpour *et al*., 2018). Thus, in this study, turmeric extract with various solvents was able to suppress hatching of Meloidogyne eggs by at least 60% with methanol-soluble turmeric extract, 46% with n-hexane-soluble turmeric extract, and 35% with water-soluble turmeric extract.

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Effect of Vegetable Extracts on Penetration Meloidogyne spp.

In addition to its effect on inhibiting the hatching of *Meloidogyne* spp eggs, turmeric extract also suppressed the infectivity of stage 2 of *Meloidogyne* larvae. The number of Meloidogyne larvae that penetrated the treatment with vegetable extracts was significantly less than the number of larvae that penetrated the control. This can be seen in the number of shoots formed on the roots of tomato plants that were treated with vegetable extracts, which was much less than the number of shoots formed on the roots of tomato plants that were not treated with the extract (control).

Once hatched, the larvae of stage 2 (J2) are attracted to the tips of the roots in the elongation area and on the lateral plane of the roots. Nematodes are attracted to the root tips by CO2, and perhaps by small, invisible molecules of amino acids and other substances. J2 Penetration into the elongation zone by a mechanical system (stylet) that requires energy and chemical mechanisms (cellulase and pectinase). After penetration, the larvae move between the root bark and the wood via cortical cells between the root tips, namely the meristems and and migrate back to the vascular cylinder in the elongation zone of the cells. In connection with this, in addition to interfering with energy formation in nematodes (Shakya & Yadav, 2020), the active ingredients contained in ginger, turmeric, and galangal are also thought to interfere with the sensory nervous system of *Meloidogyne*.

Some alkaloids and tannins can be nematicidal. According to (Liu *et al*., 2020), the use of plant extracts containing alkaloid compounds can inhibit the development of nematodes. Alkaloids are also nematicides that can inhibit the rate of metabolism in the nematode body. Tannin compounds are also able to precipitate proteins. The effect of tannins on the larval skin cell wall is that it can block the nematode muscle response and react with acetyl choline so that the nematodes are paralyzed and die. (Sepahpour *et al*., 2018) stated that tannins can inhibit the enzymatic system of nematodes and react with proteins that make up cells so that it can reduce the ability of nematodes to infect roots. This affects the formation of root cavities.

In this study, all treatments with vegetable extracts were significantly able to suppress the number of puru that occurred the most in the control. This means that in the treatment using turmeric extract with various solvents, the number of larvae that penetrated was in the low category. The three extracts used in this study contain nematicidal compounds such as alkaloids and tannins that can interfere with the nematode nervous system, making it difficult to identify the host plant.

Effect of Vegetable Extracts on the Development and Reproduction of Nematodes Meloidogyne spp.

During the life cycle of *Meloidogyne* spp., there are phases that occur outside the roots (hatching and penetration) and those that occur inside the roots (development and reproduction). The results of this study indicate that the active ingredient of turmeric extract does not affect the development and reproduction of *Meloidogyne*. The diameter of the bladder and the number of egg sacs (as well as the number of eggs) which respectively represent the development and reproduction of *Meloidogyne*, there were no significant differences between all treatments and controls.

The biochemical reaction of plants to root-knot nematode infection is the occurrence of hypertrophy and hyperplasia. Hypertrophy is a condition that shows the size of cells in the tissue increases. Hyperplasia is a condition that shows the number of cells in the tissue increases. Furthermore, the stage II larvae of *Meloidogyne* will attack the meristematic root

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tips. The cells will continue to divide and the division is controlled by IAA compounds. When nematode larvae attack plants, protease enzymes are secreted from the subdorsal glands, which break down proteins into amino acids. One type of amino acid that results from the breakdown is tryptophan which is a precursor to the formation of IAA. With more and more IAA is formed resulting in increased cell division. Therefore, the plant will form larger cells.

In this study, the mechanism of purulent formation did not occur. Based on the relatively small size (diameter) of the lungs and the small number of egg masses, especially in the control, there were indications that the tomato varieties used in this study showed resistant reactions. In the control, the size of the shell should be large with the number of egg masses also approaching the number of shells. However, the results showed that the size of the bladder was very small and the amount of mass formed in the control was less than the number of bladders. The resistance reaction of plants is also reinforced by data on the average number of very few eggs. The number of eggs in the control was about 80 compared to 500-2000 eggs formed in sensitive plants. All of galls formed are very small.

The increase in concentration in each extract cannot be said to be directly proportional to the high inhibition of nematode development *Meloidogyne*, the difference in concentration of each extract is fluctuating because the range between concentrations is too close so that the response to the effect of treatment is relatively the same. This is influenced by the response of the plant itself to the formation of IAA. This is in accordance with the theory which states that the actual purpose of this gall formation for plants is to inhibit the movement of nematodes in the tissue. In addition, this can also be due to the tomato cultivar being resistant (to NPA) which is thought to contain Phenylalanineamonia lyase (PAL). According to Shakya & Yadav (2020) an increase in PAL activity in plant roots led to the synthesis of more phenolic compounds which in turn prevented the development and reproduction of nematodes expressed in the diameter of the galls.

Conclusions and Suggestion

Vegetable nematicide extracts with various solvents were effective in inhibiting the activity of nematodes outside the roots but had no significant effect inside the roots (penetration and reproduction). To prevent hatching of eggs in the soil and penetration of J2 larvae into the roots, turmeric extract was applied at planting time 30 day after plant, then every week.

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