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Effect of *Lantana camara* L. and *Parthenium hysterophorus* L. to Control Pathogenic Nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood

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ABSTRACT

Lantana camara L. and *Parthenium hysterophorus* L. are two invasive weed species in Malaysia, but sufficient information is not available on the uses of these invasive species for producing value-added products. Therefore, the plant extracts of these species were tested against the pathogenic nematode, *Meloidogyne incognita* to explore the possibility of using these species in the industry for commercial production of bionematicide. Aqueous extracts of the weed species were made at the Universiti Malaysia Kelantan (UMK) laboratory by mixing 0 g, 10 g, 20 g, and 40 g dried plant powder with 100 ml distilled water. The plant extracts (6 ml) at four different concentrations, e. g. 0%, 10%, 20% and 40% were added to the nematode suspension containing 50.33±2.52 Juveniles in urine jars, and the contents were kept undisturbed for 24 hours. The number of dead nematodes was counted by placing the treated extracts on the microscopic slide and was observed under a compound microscope. The data revealed that both the species had the killing effect on the nematode. Between two plant species, *L. camara* was more effective causing 83% mortality at 40% concentration of extracts. *P. hysterophorus* caused 81.5% mortality of the nematode at the same concentration (40%). The plant species might be the raw materials for producing bionematicide in the industry, and the effective concentration might be reduced by purifying and partitioning the crude extracts with appropriate solvents and techniques.

Keywords: Bionematicide, *Lantana camara*, *Meloidogyne* incognita, *Parthenium hysterophorus*, root-knot nematode

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INTRODUCTION

Lantana camara is an invasive weed and a shrub usually found on the roadside and fallow land, but sometimes it is used as a flowering ornamental plant. The plant is reported to have medicinal properties such as antibacterial activity, cytotoxic activity, antifertility, antifungal activity, anti-inflammatory activity, antimotility activity, antidiabetic activity, larvicidal activity, antioxidant activity, wound healing activity and hepatoprotective effects (Ghisalberti, 2000; Kalita et al., 2012).

Parthenium hysterophorus is another invasive alien species in many countries of Asia, Africa and America (Seema, 2011). It has many negative impacts on human and animal health, crop production, and biodiversity. In Malaysia, the weed has been identified as an invasive, allergenic and environmental pollutant (Karim, 2014; Karim et al., 2017). However, the weed is reported to possess some medicinal and pesticidal properties as well (Kumar et al., 2013).

Most of the nematodes are well known as a plant-parasitic organism and the species, *Meloidogyne incognita* is an important plant-parasitic nematode which affects the quality and quantity of the crop production including banana, tomato, pulse and many vegetables. This nematode multiplies mainly due to the completion of several generations within a single growing season. According to Al-Hazmi et al. (2017), the species, Meloidogyne are responsible for the reduction of crop yield by 10% in vegetable crops. In some places the crop losses extend to 30%, depending on the host cultivar, population density and the species of nematode that involves. Therefore, bio-control of this parasite is explicitly essential for sustainable crop production (Stoffelen et al., 1999). The usual practice of control is the use of artificial chemical, e.g., Methyl bromide, which leads to environmental pollution. The use of botanical to control this pest is more eco-friendly and sustainable. Udo et al. (2014) studied the effect of *Paecilomyces lilacinus* in combination with *Lantana camara* leaf extract in controlling root galling of tomato where they noticed that *L. camara* with *P. lilacinus* were effective in reducing egg production of *M. incognita*. However, the killing effects of the extract of *L. camara* on the *M. incognita* extracted from banana were not targeted in their study. The plant extract of *L. camara* and *P. hysterophorus* are allelopathic and might be effective botanicals against the root-knot nematode. Unfortunately, sufficient information on this aspect is not available in Malaysia.

The objective of the study was to assess the nematicidal effect of plant extracts of *L. camara* and *P. hysterophorus* on *M. incognita.*

MATERIALS AND METHODS

The leaves of *Lantana camara* were collected from the roadside of Bera, Pahang while that of *Parthenium hysterophorus* were collected from Baling, Kedah. The nematode, *M. incognita* was collected from University of Malaya (UM) where these were reared in the experimental soil

of banana plantation. The infected banana plants along with contaminated soil were carried to Universiti Malaysia Kelantan (UMK) AgroTeck Park, Jeli Campus. The collected banana plants were grown at AgroTeck Park until the Juveniles were used in the study.

The nematodes along with root soil of infected banana plants were collected from AeroTech Park. The soil around the banana suckers contained the test nematodes, *M. incognita.* The 'Baermann techniques' was used for extraction of active nematodes from the soil. The soil was removed from the roots of the banana plants, and a container was used to extract the nematode. A small handful of infected soil was overlapped on two layers of wet facial tissues. The wrapped soils were placed in a small porous dish on the top of a mesh. Water was added gradually so that the mesh is slightly covered with water and the soil become wet. After 24 hours rest, the nematodes were crawled out of the soil. The container was covered with plastic wrap to prevent from drying. The bundled soil was removed from the container, and the water in the container was examined using a compound microscope. Before using the nematode suspension for testing against the plant extracts, the number of nematode per unit volume of suspension was counted following the standard procedure of Chedekal (2013). Ten ml of suspension was put into a beaker and drop by drop were examined under a microscope until the ten ml of suspension was finished. The presence of nematodes in the suspension drops was confirmed by seeing the thread-like mobile organism in the suspension. This step was replicated thrice. The average number of nematode in 10 ml of suspension was 50.33 ± 2.52.

The early collected fresh leaves of *L. camara* and *P. hysterophorus* were put in paper bags. The collected leaf samples in paper bags were dried in an electric oven at a temperature of 30ºC for three days (Maharjan et al. 2007). The dried leaves were ground into a fine powder using a mechanical grinder. The ground samples were sieved to get a fine powder. The aqueous extract was prepared using distilled water with specified amount of powder, e.g. water (10 g in 100 ml) made 10% solution and so on. After mixing the sample with distilled water, all the solutions were placed into an orbital shaker for 24 hours. Then the extract was first filtered through a clean muslin cloth and then through a filter paper. The filtrates were kept in a refrigerator at 4ºC until it was used for the experiment.

The test was carried out in urine jars. There were 24 urine jars in four replications (2x3x4=24). Ten ml of suspension was taken in a urine jar which contained about 50.33 nematodes. Six ml of early prepared plant extracts of different concentrations of two weed species was added to the suspension in the jars. All the urine jars were kept at ambient temperature. After 24 hours of incubation, all dead and alive 2nd stage juveniles (J2) were counted with the aid of an inverted compound microscope at a magnification of 100x. The dead juveniles attained the shape of a straight line or a bit curved, and the mortality was ensured by

touching the juvenile with a fine needle. The percentage of mortality due to the effect of plant extracts was estimated according to the formula as below:

$$\text{Mortality (\%)} = \frac{\text{(No. of live nematode before treatment)} - \text{(No. of live nematode after treatment)}}{\text{No. of live nematode before treatment}} \times 100$$

The treatments were arranged in a Randomized Block Design, and the data were analyzed using two-way ANOVA by using computerized statistical program SPSS to see significant differences between the plant extracts and their concentrations. The Turkey test was used to compare the means of the treatments at 5% level of significance (Pallant, 2011).

RESULTS AND DISCUSSION

The two-way ANOVA indicates that there was no significant difference between plant species in controlling the nematode, $F\ (1, 20) = 2.840,\ p = 0.107$. Significant difference was noticed among the concentrations, $F\ (3, 20) = 1\ 438.12,\ p = 0.00$. No significant interaction between two factors was also found in the study, $F\ (3, 20) = 0.356,\ p = 0.785$.

It is evident that the application of plant extracts of selected plant species made the significant death of the nematode. All the treatments exhibited nematicidal potential of varying degrees. The mortality of juveniles might be due to nematicidal chemicals present in the leaf extract as *L. camara* contains camaric acid and olenolic acids which may have larvicidal or ovicidal properties (Ghimire et al., 2015). When the dead nematodes were studied under microscopes, it became apparent that they had a variety of shapes. The shape of nematodes before treatment application and after the application was different. Before the application, the shape of nematode was curled (∞-shape) or sigmoid (∑-shape) and bent (banana-shape) while after the treatment application the nematodes were like straight lines (I-shape) and bent banana-shape (Figure 1).

On an average, more than 54% of the nematodes were killed due to treatment with the plant extracts. It means that more than half of the nematodes were died due to the treatments. The number of dead nematodes at 10% concentration of *L. camara* and *P. hysterophorus* were 21.5 and 20.0. The percentage of mortality at 40% concentration for *L. camara* and *P. hysterophorus* are 83.0 and 81.5 (Table 1). The leaf extract of *L. camara* at 100% concentration exposed for 48 hours was found effective in controlling *Meloidogyne* juveniles in Nepal (Ghimire et al., 2015). The nematicidal activity of *L. camara* against juveniles of *Meloidogyne* spp. has also been reported by many authors (Begum et al., 2008; Qamar et al., 2005).

The mortality of the nematode was concentration dependent, and the mortality percentages increased linearly with the concentration. Based on Figure 2,

40% concentration of *L. camara* and *P. hysterophorus* gave the highest mortality compared to other concentrations. The allelopathic plants at higher concentration produced the greater phytotoxic effect on the nematodes.

Figure 1. Characteristics of nematodes before and after extract application

Table 1
Number of live nematodes before and after applying of plant extracts

Concentration of plant extracts	***Lantana camara***			***Parthenium hysterophorus***			**Mean Mortality**
	Before	After	Mortality % ± SD	Before	After	Mortality % ± SD	
0%	50	50	-	50	50	-	-
10%	50	39.25	21.50 ± 1.92	50	40.0	20.00 ± 1.63	20.75
20%	50	18.50	63.00 ± 2.58	50	19.8	61.00 ± 3.46	62.00
40%	50	8.50	83.00 ± 2.58	50	10.0	80.00 ± 2.82	81.50

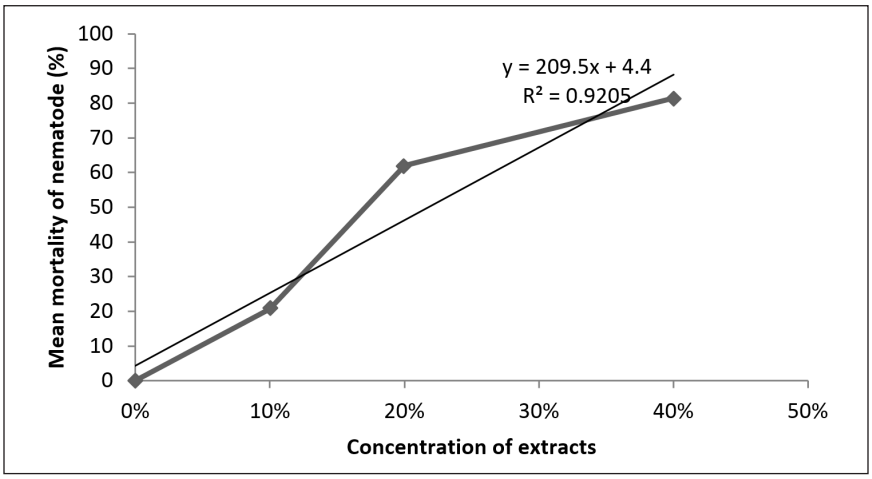

Figure 2. Effect of concentration of extracts on nematode mortality. The values are mean of two weed species (The straight, thin line indicates the linear relationship)

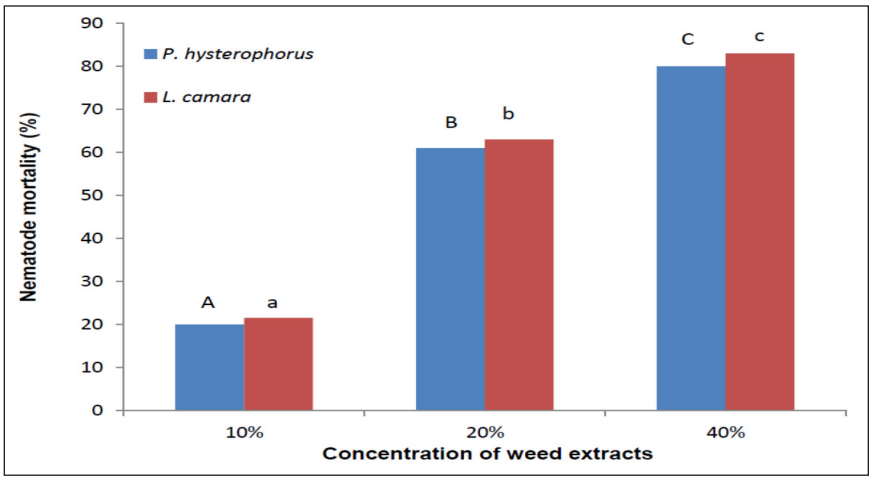

Figure 3. Effects of weed species and different concentrations on the nematode mortality (Dissimilar letters above the red and blue bars indicate significant differences between same colored bars)

The highest nematode mortality (83%) was noticed due to the application of 40% extracts, and there was no significant difference between the plant species at the same concentration (Figure 3). From Figure 3, it is evident that *L. camara* is a bit more effective than *P. hysterophorus* in killing the nematodes. The plant *L. camara* caused 15.9% more mortality than *P. hysterophorus.*

Ghimire et al. (2015) also observed similar kind of killing effect of *L. camara* on *M. incognita*. They stated that the extraction from leaves of *L. camara,* and leaves and root of Mexican marigold could reduce the hatching of *M. incognita* eggs. The inhibitory effect of extracts of the selected botanicals including *L. camara* and *P. hysterophorus* might be due to the

chemicals present in the extracts that possess ovicidal or larvicidal properties. Probably these chemicals affected the embryonic development of the nematode or killed the eggs (Wondimeneh et al., 2013). *L. camara* contains pentacyclic triterpenoids, e.g. coumaric acid, lantanilic acids and olenolic acids which may have larvicidal and ovicidal properties (Ahmad et al., 2010). The properties of *L. camara* and *P. hysterophorus* are also reported to have insecticidal, nematicidal and herbicidal effects in India (Datta & Saxena, 2001; Mishra, 2014).

Wondimeneh et al. (2013) suggested that the nematicidal properties of botanicals were dependent on plant species, plant growth stages, application method and the species of nematode tested. The nematotoxicity of the plant is increased with increase in concentrations and the time of exposure to the nematode. Chaudhary et al. (2013) tested eleven weed species including *L. camara* in Eritrea and observed the least inhibitory effects of *L. camara* on egg hatching of *M. incognita*.

Both the species of plant exhibited toxicity towards the juvenile of the root-knot nematode, *M. incognita*. Usually, when the concentration of plant extract increases the number of nematode mortality also increases. In this study, more than 83% mortality was noted due to 40% concentration of *L. camara*.

However, no concentration greater than 40% was used in this study, and we do not know what could happen if we used more than 40% concentration. But 83% control is an indication of effective control which we could achieve with 40% concentration of the plant extract. More studies should be conducted on the effect of different solvents examples ethanol, methanol, etc., which might give varying degrees of killing effects due to the isolation of different amounts of allelochemicals. The effects of mixing of various plant extracts to control the nematode should also be studied. The blending of plant extracts may provide with a combination of more allelochemicals, which could bring more mortality.

CONCLUSION

In conclusion, the plant extracts of *L. camara* and *P. hysterophorus* can be used to control the plant parasitic nematode *M. incognita*. Using 40% concentration of aqueous extracts of the weeds for more than 80% control of root-knot nematode seems effective botanicals. To produce bionematicide from these weed species the crude extract should be purified and made free from other non-effective compounds, and in that case, a lower concentration of the extracts may be sufficient to control the nematode effectively. The use of these invasive weeds for developing nematicide has explored the possibility of control of the weeds by utilization. The harmful plants could be converted to value-added product. The possibility of establishing SMEs in the country with this technology has also explored a new direction of agro-business.

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