Incidence of Root-Knot Nematode on Misai Kucing (*Orthosiphon stamineus)* in Peninsular Malaysia: A New Discovery

ABSTRACT

A survey was conducted over one and half years to estimate the occurrence of root-knot nematode infestation on *Ortosiphon stamineus* (misai kucing) in selected locations around Peninsular Malaysia. Infestations were monitored in ten misai kucing plantation areas in Pulau Pinang, Perak, Negeri Sembilan, Selangor, Johor, Terengganu, and Kelantan. The survey indicated that misai kucing grown on sandy clay-type soil tends to be more susceptible to heavy root-knot nematode infestation. The highest frequency of disease incidence in which almost all the roots of misaim kucing have a knot-like appearance (100%) was reported from the Batu Kurau area. The other two locations, Ipoh and Pantai Baru, had mild infestations, and these were reported as 16% and 4% incidence. The methodology for access the damage caused by the root-knot nematodes in misai kucing, *Meloidogyne* gall index and its egg-mass index were calculated and these were found in the range of 1-4. The average egg mass from ten randomly selected stunted misai kucing plants from Batu Kurau was recorded as 599.4, with the highest number of 2274 egg mass per plant. Sampled plants showed the significant presence of *Meloidogyne incognita*. The root-knot nematodes were morphologically identified based on the perineal patterns of females and the head morphology of both *Meloidogyne* females and males.

Keywords: Meloidogyne spp.; misai kucing; Orthosiphon stamineus; root-knot nematode.

1. INTRODUCTION

Among Southeast Asian people, misai kucing is a popular traditional remedy in the form of herb or coffee mix for treating rheumatism, diabetes, hypertension, tonsillitis, epilepsy, menstrual disorders, kidney stones, lithiasis, body aches, edema, gout, pain stomach, hot fever, influenza, gonorrhea, hepatitis, syphilis, jaundice and kidney inflammation and bladder [31. Misai kucing was also well-known for its stronger diuretic effect than other natural herbs [2]. In Europe, according to Indubala et al. [3] misai kucing herbal drink is also called Java tea. Since 1930, numerous studies have been carried out on the importance of misai kucing in Indonesia, Malaysia, Vietnam, and Myanmar [4]. A total of 116 phytochemical components were isolated from misai kucing plants, such as monoterpene, diterpene, triterpene, saponin, flavonoid, essential oils, and organic acids. Some of the isolated components show antioxidant, antimicrobial, anti-obesity, and hepatoprotective activity [4].

Misai kucing (*O. stamineus*) is a species of herbaceous shrub found in Africa and Southeast Asia that grows up to a height of 1.5 meters. It can also be found abundantly in tropical regions of South China, India, and Queensland. The words *Orthosiphon* and *stamineus* are derived from Latin, respectively, which means a straight tube and stripes like mustaches on the flower. It is a type of flowering plant and is often planted as an ornamental tree. The leaves are green in color, ellipse or rhombus, with a smooth surface and arranged in opposite pairs, short, about 0.3 cm long. The stem of misai kucing is rectangular, reddish-colored, growing upright, and branching. The flower has a vertical about 16 cm long and long filaments such as mustaches. The flowers are white for white varieties and purple for purple varieties. According to Lee et al. [5], purple varieties have more bioactive components than white varieties, but white varieties received more focus in many research.

Nematodes are a major agricultural pest worldwide, affecting a wide variety of crops, including vegetables, fruits, and ornamental plants. Root-knot nematodes are one of the majors in these genera (*Meloidogyne spp.*) [6]. These nematodes are microscopic, parasitic worms that infect the roots of plants, causing the formation of characteristic galls or "knots" on the roots [6]. Root-knot nematodes may cause plants to die in severe infestations. The galls formed by root-knot nematodes disrupt the plant's ability to absorb water and nutrients, leading to stunted growth, wilting, and reduced yield [7]. These nematodes have a wide host range, affecting over 2,000 different plant species, making them a significant threat in both agricultural and horticultural settings [8]. Many farmers struggle to manage and control this pest. Control of root-knot nematodes is challenging and often involves a combination of methods, including crop rotation with non-host plants, the use of resistant plant varieties, soil fumigation, and biological control agents like nematode-trapping fungi [9].
Misai kucing is always categorized as plants that are free from infection by pests and diseases. However, for the first time in 2009, this plant was reported infected by a serious attack by a lace bug in Subang Jaya [10]. Disease-wise information and records of diseases on this plant are currently unavailable. Therefore, urgent consideration to cover this disease information is needed. The present study explores for the first time one of the most serious problems facing misai kucing in Malaysia, which is an infestation of root-knot nematodes.

2. MATERIALS AND METHODS

The survey was conducted in 10 different locations throughout Peninsular Malaysia. The areas surveyed were Negeri Sembilan (Pantai and Sendayan), Pahang (Raub), Perak (Taiping and Ipoh), Johor (Pontian), Pulau Pinang (Kepala Batas), Terengganu (Dungun), Kelantan (Bachok) and Selangor (Serdang). Isolation, identification of pathogens, and morphological characteristics of disease samples were taken from trees. Disease samples were collected from survey sites around Penang, Pahang, Terengganu, Selangor, Perak, Negeri Sembilan, Johor, and Kelantan. All samples were taken to the laboratory to isolate disease pathogens.

2.1 Nematode Infestation Survey

Misai kucing grown in ten selected locations was surveyed during the dry season April-September 2016/2017 and the wet season October-March 2016/2017. The observations on the symptomatology disease incidence and severity of root-knot nematode, disease incidence, and severity were recorded on misai kucing plants in the field. The data on disease incidence (DI) were recorded using the absent (-) and present (+) technique, while data on disease severity index (DSI) were recorded as described by Mamza et al. [11] index (Table 1).

The percent disease index (PDI) was calculated using Equation 1 as follow.

$$PDI = \frac{\text{Total number of plant infected}}{\text{Total number of plants surveyed}} \times 100$$

Nematode sampling was collected from the affected plants and the soil around the roots. These were then taken to the laboratory for further analysis to determine the identification of the nematodes and possibly their density.

2.2 Nematode Egg Masses Staining

Phloxine B stains the gelatinous matrix surrounding Meloidogyne eggs, increasing the visibility of the egg masses and enabling a rapid count of adult female nematodes and egg masses. The solution is made by adding about 15 mg of Phloxine (B) to 500 mL of distilled water. The rinsed root was placed in a jar containing Phloxine B solution for about 30 minutes to one hour. The following data were taken and recorded; 1) Number of egg masses, and 2) gall score [12].

2.3 White Tray Technique for Nematode Identification

Two hundred grams of soil was used for the extraction of nematode by using the Whitehead tray method [13]. An ordinary stainless-steel tray was used on the whitehead tray. Plastic mesh was placed on the tray, and a single piece of tissue paper was placed on it. The soil was spread on the tissue paper. About 200 ml of water was poured, just touching the soil. The trays were left to stand for 24 hours while nematodes dropped into the tray from the soil. The nematode-water suspension was poured into a clean beaker and left to stand for 3 hours to concentrate the nematodes in manageable volume water. The excess water was carefully siphoned out. The nematodes were then uniformly distributed in the remaining liquid by blowing with a pipette. One milliliter of the nematode suspension was withdrawn from the beaker, and nematodes were counted and examined under a stereo microscope.

Table 1. The disease severity index proposed by Mamza et al. [11]

Disease severity index	Description
1	No symptom
2	1-25% total leaf number with symptoms
3	26-50% total leaf number with symptoms
4	51-75% total leaf number with symptoms
5	76% or more total leaf number with symptoms

3. RESULTS AND DISCUSSION

Surveys were conducted in ten locations of misai kucing plantations including Serdang, Ipoh, Tasek Gelugur, Pasir Raja, Pantai, Taiping, Bachok, Pontian, Sendayan, and Raub from Mac, 2016 until December 2017 for both dry and wet season to record the incidence and severity of stem rot, leaf spot, leaf scorch, virus and discoloration and also infestation of nematode as described by Mohd Nazri et al. [14]. The data on the incidence and severity of nematode infestation at different locations were recorded and are present in Table 2. Nematode infestation was observed in only three places: Ipoh, Pantai, and Taiping, with disease incidence percentages of 16.0 percent, 4.0 percent, and 100.0 percent. However, the disease severity of this infestation was quite severe at the Taiping plantation, with an index value of 3.36, whereas all the plants already had galls on the root. Among these three soil conditions observed in Table 2, misai kucing resistance to root-knot nematode found to be very was poor in bris and sandy clay soil, as Taiping recorded the highest incidence of nematode infestation of 100.0 percent with a high severity index of 3.36. However, the result was very contrary to the Bachok plantation as there was no infestation of nematode at all in Bachok bris soil. This result may happen as the cultivar of the same crops shows different types of response and is influenced by various factors such as temperature, planting time, origin of plants, and crop rotation, all of which could affect the survival and pathogenicity of the nematode pathogens as described by Roberts and Caswell [15]. These also included the soil's compactness and the availability of aeration and moisture within a field. Kim et al. [16] study also reported that gall and egg-mass formations were remarkably increased in bed-soil and sand mixtures, which coincided with the general aspects of severe nematode infestations in sandy soils. The reports also suggested that sandy soil conditions, which provided sufficient aeration, leading to their mobility increased for the enhanced root penetration, positively affect healthy plant growth, thus enhancing nematode growth and feeding activities.

Root galls are caused mostly by the root-knot nematodes *Meloidogyne spp*. [17]. All the symptoms observed on misai kucing roots are the same as root galls described in the literature [17]. Fig. 1 (a & b) and Fig. 1 (c - e) show visual observations and microscopic view using a stereo microscope. Fig. 2 (a - h) shows the microscopic view using a light microscope. Male *M. incognita* showing stoma with stylet (Fig. 3). The head shape of *M. incognita* male is very characteristic as the labial disc is dumbbell-shaped, large and round, centrally concave, and raised above the medial lips. The stylet is blunt and broader than the medial portion of the cone.

Fig. 1. Nematode infestation. The Misai kucing plant on the left side has normal leaves, and the root hairs are still abundant in the primary root main stem. In contrast, the Misai kucing plant on the right side shows small leaves with little root hairs remaining on the primary roots, showing a severe nematode attack (a). Root galls can be observed clearly from this photo (b). Adult female of *Meloidogyne spp.* and egg masses under stereo microscope (c). Egg masses on misai kucing roots turn red after staining with Phloxine B (d). A close-up view of egg masses (red) of *Meloidogyne spp.* attach on roots (e)

Table 2. Prevalence disease incidence in percentage and disease severity index of misai kucing grown in ten plantations in different states of Malaysia from 2016-2017

Location	Serdang	Ipoh	Tasik Gelugur	Pasir Raja	Pantai	Sendayan	Raub	Taiping	Bachok	Pontian
Type of soil	Mineral soil	Mineral soil	Mineral soil	Mineral soil	Mineral soil	Mineral soil	Mineral soil	Bris, sandy clay soil	Bris, sandy clay soil	Peat soil
Disease Incidence (DI)	0.00	16.00	0.00	0.00	4.00	0.00	0.00	100.00	0.00	0.00
Disease Severity Index (DSI)	1.00	1.38	1.00	1.00	1.04	1.00	1.00	3.36	1.00	1.00

Table 3. Nematode isolation analysis result

Host plant	Sample no.	Diagnosis
Misai kucing	1	*Meloidogyne sp.* (rate of 200/200 gm soil)
Misai kucing	2	*Meloidogyne sp.* (rate of 266.6/200 gm soil) and *Helicotylenchus sp.* (rate of 100/200 gm soil)
Misai kucing	3	*Meloidogyne sp.* (rate of 133.3/200 gm soil)
Misai kucing	4	*Meloidogyne sp.* (rate of 233.3/200 gm soil)
Misai kucing	5	*Meloidogyne sp.* (rate of 166.6/200 gm soil) and *Helicotylenchus sp.* (rate of 33.3/200 gm soil)
Misai kucing	6	*Meloidogyne* sp. (rate of 66.6/200 gm soil)

Fig. 2. Nematode: The egg mass of *M. incognita* looks broken and produces J2 (a). Condition of eggs of *Meloidogyne spp*. in the egg mass (b). Egg of *Meloidogyne spp.* (c). J1 in egg (d). Egg size difference J1 and J2 (e). Basal knob on male (J2) *Meloidogyne spp*. (f). Anus J2 (g). Basal knob on female *Meloidogyne spp*. (h)

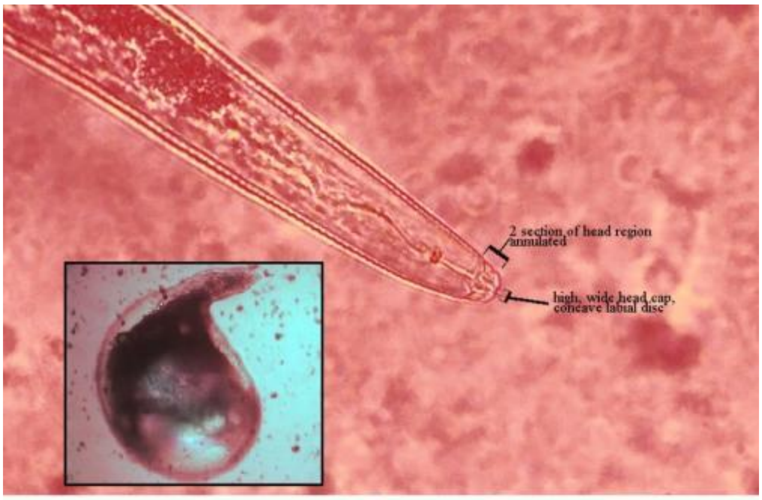

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Fig. 3. A male of *M. incognita* showing a stoma with a stylet. The head shape of *M. incognita* male is very characteristic as the labial disc is dumbbell-shaped, large and round, centrally concave, and raised above the medial lips. The stylet is blunt and broader than the medial portion of the cone. A small photo shows a female *M. incognita* body

Nematode extraction analysis was used to determine the precision of root-knot nematodes. Table 3 shows the frequent precision of *Meloidogyne sp.* in six sample analyses. However, other species of nematode

(*Helicotylenchus sp.*) were also detected, but fewer per gram of soil.

4. CONCLUSION

In conclusion, the root-knot nematode found infesting misai kucing's roots was identified as *M. incognita*. The presence of this infestation in the farmers' plots might cause yield loss. Soils such as clay, sandy, and bris soil are ideal environments for this root-knot nematode, which will serve as a warning to farmers to take preventative measures against the disease.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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