Investigation of Rice Root Nematode *Hirschmanniella oryzae* (Luc and Goodey, 1964) in Pyarkatat Cultivated Rice fields at Hinthada Township

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Abstract

Rice is one of the most important staple foods for more than half of the world's population, especially in Myanmar. Plant-parasitic nematode is one of the pests for rice production. The present study was carried out to know the infestation of plant nematode in soil and root samples of different varieties of rice plant collected from cultivated field of eight villages namely Khanwe, Pyarkatat, Moegoke, Taunngu, Sabalsu, Pyarkatat East, Pyarkatat West, Pyarkatat South villages during September 2015 to January 2016. The collected rice varieties were Sinthukha, Shwewarsan, Yarkyaw, Hmawbi and Manawbaykyar. The investigated rice root nematode, *H. oryzae* were recorded by photograph under compound microscope. The result was found out that all rice varieties were infested with the rice root nematode *Hirschmanniella oryzae*. Among them, the average *H. oryzae* population in roots was the highest in Shwewarsan and Sinthukha varieties from Pyarkatat and Sabalsu villages whereas the population of nematodes in soil was found in Yarkyaw variety from Moegoke village.

Keywords: Rice, *Hirschmanniella oryzae,* investigation

Introduction

Rice is the main cereal crop in Myanmar. Agriculture in Myanmar, dominated by paddy rice cultivation, generates a direct or indirect economic livelihood for over 75% of the population. Rice is grown throughout the country by resource poor rural farmers and landless agricultural labourers on small farms averaging only 2.3 ha in size (Okamoto, 2004). The total number of rice growing area is 6.4 million ha or 60% of the total cultivated area. Monsoon rice and irrigated summer rice are grown on 4 and 2 million ha, respectively, mainly in the lowlands (New New Tun, 2006). Rice pests include weeds, pathogens, insects, nematodes, rodents, and birds.

Nematodes are extremely abundant and diverse animals; only insects exceed their diversity. Nematodes were noted early in human history because some serious human diseases are caused by relatively large vertebrate-parasitic nematodes. More than 25,000 species of nematodes have been described up to the present, of which around 4,000 are marine free-living nematodes, 6,000 terrestrial free-living nematodes, 12,000 parasitic nematodes of vertebrates, and 3,500 parasitic nematodes of invertebrates (Poulin and Morand, 2000; Hugot *et al*., 2001).

Today parasitic nematodes threaten the health of plants, animals and humans worldwide. Some, however, are serious human, animal, and plant pathogens that attack animals or humans, but do not attack plants. Heartworm in dogs and cats and elephantiasis in humans are examples of nematode diseases in animals and people. All crops are damaged by at least one species of nematodes. **N**ematode infections have a widespread distribution being found in both Temperate and Tropical climates (ANSI, 2004).

Plant-parasitic nematodes are found to be harmful in rice cultivation. Among the plant-parasitic nematodes, *Hirschmanniella oryzae*, i.e.Rice Root Nematode (RRN), is among the major pests of rice and is the most common plant-parasitic nematode found on

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irrigated rice. Recent modifications in cultivation practices have lead to a substantial increase in rice production, which has been accompanied by heightened levels of RRN. The proportional increases in RRN with rice production can be explained by the nematode's impeccable adaptation towards constantly flooded conditions in which irrigated rice is often being grown (Bridge *et al*., 2005). RRN-infected roots first show a yellowish to brown color that eventually darkens, and heavily infected roots may decay after turning brown or black. These below ground symptoms begin by the formation of small brown lesions at points where nematodes have ruptured the surface and entered. Following these early symptoms, damaged epidermal cells may become necrotic and cavities may form inside the roots as a result of damaged cortical cells (Ichinohe, 1972).

There are many constraints, both abiotic and biotic, that can reduce rice yield and seed quality. Among them, the biotic constraints were plant-parasitic nematodes (Bridge *et al*., 2005). In Myanmar, *Hirschmanniella oryzae*, *Aphelenchchoides besseyi*, *Ditylenchus angustus, Meloidogyne graminicola* and several species of other genera such as Pratylenchus, Hopolaimus, Criconemoides and Tyklenchorhynchus, have been reported from rice (Aung Swe, 1997; Po Po Than, 2003). Although no detail information is at present available on the damage and yield loss plant-parasitic nematodes are causing to rice plants and rice production in Myanmar, preliminary observations have shown that severity of these nematodes species can cause reduction in rice and seed quality (Aung Swe, 1997).

This nematode is perfectly adapted to the constant flooded conditions in which irrigated rice is often being grown in the lowlands (Fortuner and Merny, 1979). It is one of the few plant-parasitic nematodes species that can survive under anaerobic conditions (Babatola, 1981). In Myanmar preliminary observation show that it can spread from infested rice nurseries to uninfested fields and that it can cause root damage and decrease rice yield and seed quality (Po Po Than, 2003). There are also indications that *H. oryzae* is more pathogenic on the recently introduced high-yielding varieties compared with the traditionally grown cultivars (Khin Thein, 2003). The objectives of present study were to investigate the occurrence of rice root nematode *H. oryzae* in Pyarkatat cultivated rice fields and to compare the individual numbers of rice root nematodes between different collection sites

Materials and Methods

Study site and study period

The present study was conducted in eight villages namely Khanwe, Pyarkatat, Moegoke, Taunngu, Sabalsu, Pyarkatat East, Pyarkatat West, Pyarkatat South located between N latitude 17 º 30' 0" and 17 º 31' 0" and also E longitude 95 º 25' 0" and 95 º 22', Hinthada Township (Fig. 1). The study period was in the rainy rice growing season from September 2015 to January 2016.

Collection of samples

Soil and roots samples of different rice varieties viz. Sinthukha, Shwewarsan, Yarkyaw, Hmawbi and Manawbaykyar were collected from different villages. The random survey plot (15x15 ft.) was set up in each rice field and the rhizosphere soil and root samples were collected from ten plants evenly distributed in each plot at a depth of 6 inches. The collected soil and root samples were placed in plastic bags and transported to the laboratory and were kept at room temperature until the nematode extraction from the sample (Plate 1).

Procedure of nematodes extraction from soil and root samples

The nematodes were extracted from the soil and root samples using Whitehead's tray method (Whitehead's & Hemming, 1965). As the procedure, 100 g of mixed soil was spread in over a muslin cloth as a thin layer in plastic sieve (6×8 inches). The sieve was placed in a plastic tray (8×10 inches). Amount of 200 ml tap water was carefully poured from the edge of the tray until the soil layer looked wet. The root samples were washed with tap water, cut into small pieces about 1 cm long then mixed together. A 20 g of mixed root pieces was spread in over a muslin cloth as a thin layer in a plastic sieve (6×8 inches). The sieve was placed in a plastic tray (8×10 inches).

Amount of 200 ml tap water was carefully added down from the edge of the tray until the roots layer looked wet. After adding water to the tray, it is important not to move the tray. After 24 hours, nematode suspension transferred from the tray to a glass beaker then settle about 2-3 hours. Upper portion about 160 ml of suspension was discarded. Remaining 20 ml of nematode suspension was pipetted into a counting dish. On the same day, the suspensions were examined under a stereoscopic microscope and the juvenile and adult *H. oryzae* were counted. The estimation of nematode population density of *H. oryzae* was calculated for each rice variety and for each field (Plate 2).

Identification

All specimens were identified up to the species level under the compound microscope based on their outstanding taxonomic characters such as body shape, head, stylet, oesophagus, ovary, vulva position, tail shape, bursa and spicules according to Hunt *et al.* (2005).

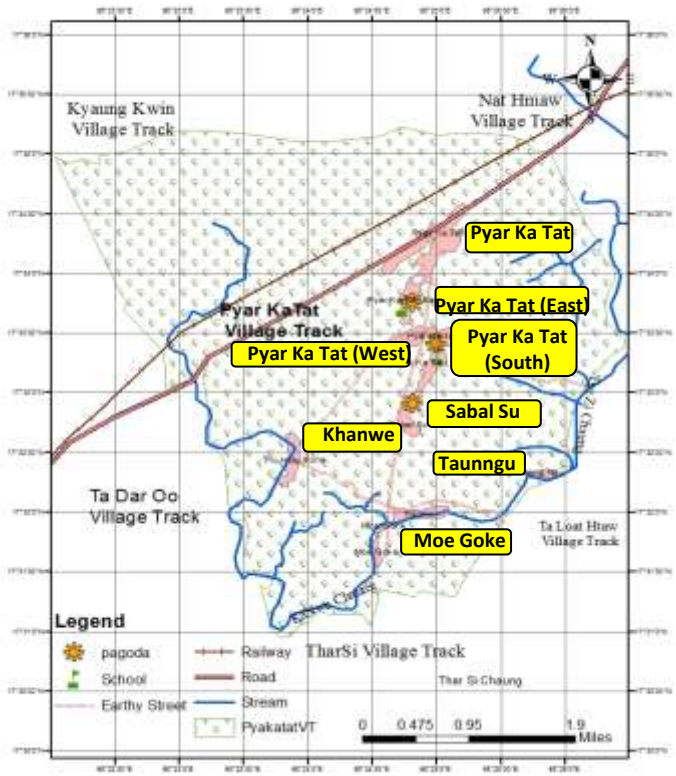


Fig. 1 Location map of studied sites

Source: Geography Department of Hinthada University

Plate 1. Collected rice specimens

A. Rinsing, weighting and cutting specimens rice plants

B. Spreading root pieces in the sieve tray C. Pouring water

D. Removing sieve E. Transferred nematode suspension F. Nematodes counting

Plate 2 Procedure of nematodes extraction in the laboratory

Results

Morphological characters

Morphological characters of the extracted nematodes were studied under the microscope and recorded with camera the images. The morphological characters were observed; elongated body, head hemispheral, stylet with well-developed basal knobs, median bulb ovoid, ventral overlapping esophagus, tail elongate conoid with mucron at tip, male with slightly arcuate spicules and terminal bursa. Male: Spicules are distinct, slightly arcuate. Tail terminus is rounded, with a ventral mucro. Female: Body is straight or slightly arctuate ventrally, head is high, hemispherical. Stylet knobs are rounded. Medium esophageal bulb is oval with elongated ventral overlap of end bulb and intestine. Ovaries are pairs; vulva median and vagina are prominent. Tail is elongated-conoid. Phasmids are small. Juvenile: Juvenile is similar to adult. It has no genital organs (Plate 4).

Systematic position of *Hirschmanniella oryzae*

Phylum - Nematoda

Class - Secernentea

Order - Tylenchida

Family - Pratylenchidae

Genus - *Hirschmanniella*

Species - *H. oryzae* Luc and Goodey, 1964

Investigation on individual numbers of *H. oryzae* in varieties of rice

Five different varieties of rice as Sinthukha, Shwewarsan, Yarkyaw, Hmawbi and Manawbaykyar were cultivated in study villages as Khanwe, Pyarkatat, Moegoke, Taunngu, Sabalsu, Pyarkatat West, Pyarkatat East and Pyarkatat South, respectively. They were Sinthukha in Khanwe, Pyarkatat, Sabalsu, Pyarkatat West and Pyarkatat South. Shwewarsan was in Khanwe, Pyarkatat, Taunngu, Pyarkatat West, Pyarkatat East and Pyarkatat South. Yarkyaw was in Moegoke and Sabalsu. Hmawbi was in Moegoke and Taunngu. Manawbaykyar was only in Pyarkatat East village. The cropping system in all villages was common rice-pea system. Shwewarsan and Sinthukha varieties were grown 37% and 31% in all collected villages. The Yarkyaw and Hmawbi varieties were cultivated the same percentage (13%), and Manawbaykyar was grown in 6 % of all collected villages. The highest and lowest growing varieties were observed Shwewarsan and Manawbaykyar respectively (Fig. 2 and Table 1).

The individual number of *H. oryzae* in Sinthukha variety was observed in soil and root samples from five villages. Among the collected villages, the highest and lowest individual numbers of *H. oryzae* were found in root samples from Sabalsu (n = 941) and Pyarkatat South village (n = 70) respectively. The highest individual number was found in soil samples from Pyarkatat village (n = 74). In Khanwe village lowest individual number of *H. oryzae* was recorded as (n = 34) (Fig. 3, and Table 2). In observation of Shwewarsan variety, the individual number of *H. oryzae* was found in soil and root samples from six villages. The highest and lowest individual numbers of *H. oryzae* were found in soil samples from Pyarkatat West (n = 126) and Khanwe village (n = 36) respectively. In root samples, the highest and lowest individual number was found in Pyarkatat (n = 1058) and Pyarkatat East village (n = 5), respectively (Fig. 4, and Table 3).

In Sabalsu village, the individual number of *H. oryzae* in root sample of Yarkyaw variety was the highest (n= 408) but the lowest individual number (n = 28) was found in soil sample. In Moegoke village, the highest individual number of *H. oryzae* (n = 248) was found in soil sample and the lowest (n = 182) was observed in root samples (Fig. 5 and Table 4). In Hmawbi variety, the higher individual number of *H. oryzae* from soil (n =154) and root (n = 691) was recorded from Taunngu village than Moegoke (Fig. 6 and Table 5). The individual number of *H. oryzae* in root sample of Manawbaykyar variety from Pyarkatat East (n = 35) was higher than the soil (12) (Fig 7 and Table.6).

In all collected rice varieties, the highest (n = 1136) and lowest (n = 47) individual number of *H. oryzae* were recorded in Shwewarsan and in Manawbaykyar variety. The lower numbers of *H. oryzae* (n = 49) was found from Pyarkatat East village. In Sinthukha variety, the highest and lowest numbers of H. oryzae were found in Sabalsu and Pyarkatat South village respectively. The highest and lowest number of *H. oryzae* from root and soil samples of Shwewarsan wererecorded from Pyarkatat and Pyarkatat East village respectively. In Sabalsu village, the individual number *H. oryzae* from soil and root sample of Yarkyaw variety was higher than the Moegoke. In Taunngu village, the highest individual number of rice root nematode was higher than the Moegoke village from Hmawbi variety. In this study, the distribution of rice root nematode *H. oryzae* was found in all collected villages (Table 7).

Table 1 Collected rice varieties from Pyarkatat cultivated fields

Villages from Pyarkatat cultivated fields	Collected rice varieties				
	Sinthukha	Shwewarsan	Yarkyaw	Hmawbi	Manawbaykyar
Khanwe	√	√			
Pyarkatat	√	√			
Moegoke			√	√	
Taunngu		√		√	
Sabalsu	√		√		
Pyarkatat West	√	√			
Pyarkatat East		√			√
Pyarkatat South	√	√			
%	31	37	13	13	6

Table 2. Individual numbers of *H. oryzae* in rice variety (Sinthukha) in different villages

Villages	No. of *H. oryzae* in soil (100 g)	Soil (%)	No. of *H. oryzae* in roots (20g)	Roots (%)
Khanwe	34	11	134	8
Pyarkat	74	25	232	15
Sabalsu	70	24	941	58
Pyarkatat West	70	23	243	15
Pyarkatat South	50	17	70	4

Table 3. Individual numbers of *Hirschmanniella oryzae* found in Shwewarsan variety

Collected villages	No of *H. oryzae*/100 g in soil	Soil (%)	No of *H. oryzae*/20 g in roots	Roots (%)
Khanwe	36	7	526	24
Pyarkatat	78	16	1058	49
Taunngu	88	18	151	7
Pyarkatat West	126	26	329	15
Pyarkatat East	44	9	5	0
Pyarkatat South	114	24	94	5

Table 4 Individual numbers of *H. oryzae* found in Yarkyaw variety

Collected villages	No of *H. oryzae* /100 g in soil	Soil (%)	No of *H. oryzae* /20 g in roots	Roots (%)
Moegoke	248	90	182	31
Sabalsu	28	10	408	69

Table 5. Individual numbers of *H. oryzae* found in Hmawbi variety

Collected villages	No of *H. oryzae* /100 g in soil	Soil (%)	No of *H. oryzae* /20 g in roots	Roots (%)
Moegoke	86	36	357	34
Taunngu	154	64	691	66

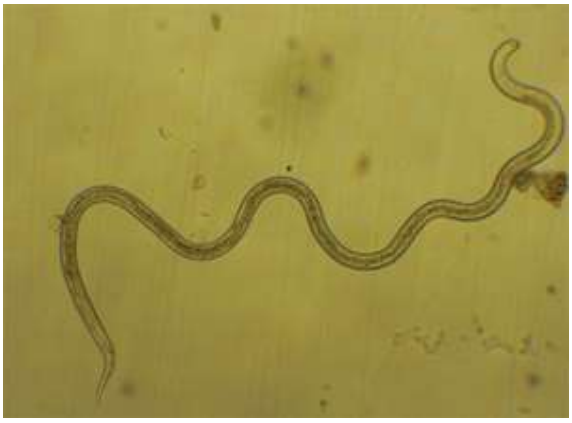
Table 6. Individual numbers of *H. oryzae* found in Manawbaykyar variety

Village	No of *H. oryzae* /100 g in soil	Soil (%)	No of *H. oryzae* /20 g in roots	Roots (%)
Pyarkatat East	12	26	35	74

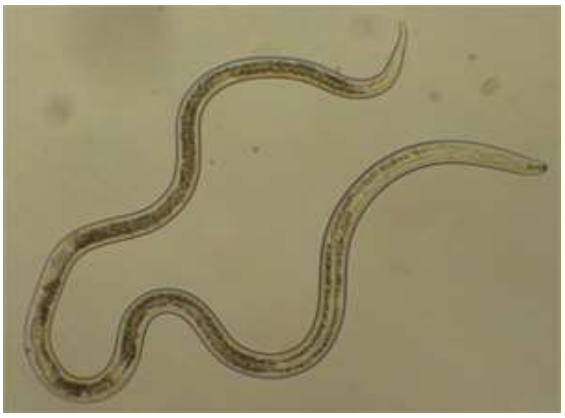
Discussion and Conclusion

A number of *Hirschmanniella oryzae* species is known as rice root nematode and endoparasite of rice plant. *H. oryzae* is distributed throughout the rice growing region of Myanmar and cause root- rot disease. Plants with the root system damaged by nematode showed retarded growth, necrosis and reduced yield. Solving nematodes problems plays an important role in improving crop yield. From the present investigation, rice roots were collected from five rice varieties such as Shwewarsan, Sinthukha, Yarkyaw, Hmawbi and Manawbaykyar. Sinthukha variety was observed from five villages. In these fields, Sabalsu village field about 58% of *H. oryzae* were infested. The lowest populations about 4% occurred in Pyarkatat South village. In other three villages were moderately infested. Mya Mya (1983) mentioned that *H. oryzae* or rice root nematode, a migratory endoparasite, was found mainly in low land area of Myanmar.

Shwewarsan variety was found in six villages from present observation. In Khanwe village field, rice root nematodes were observed 49% of six villages' fields. The number of *H. oryzae* population in soil samples of Pyarkatat south village was higher than the root. The fact was remarkable from this study period. Sampling was done at the nearly harvesting time: when the plant was inactive stage. Ying *et al*, (1997) described the horizontal distribution of *H. oryzae* in rice root, most of the population in the tip, less in the middle part and least in the basal part of the rice root Hmawbi rice variety of present study was found in Moegoke and Taunngu. The average soil and root population densities of *H. oryzae* in both Moegoke and Taunngu fields were 120/100 g soil and 524/20 g roots when the plants were in the active growth stage. Fortuner & Merny (1979) stated that the maximum root population of *H. oryzae* can be found between tillering and heading stage of the rice crop.



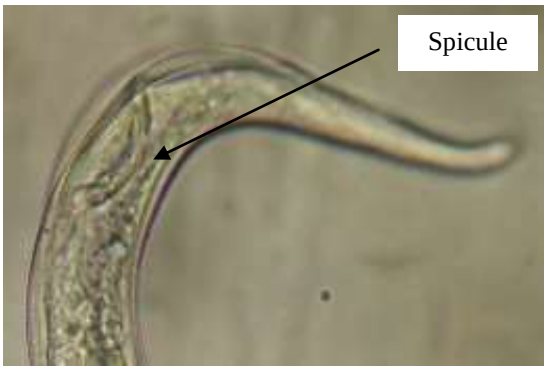
A. Male whole body



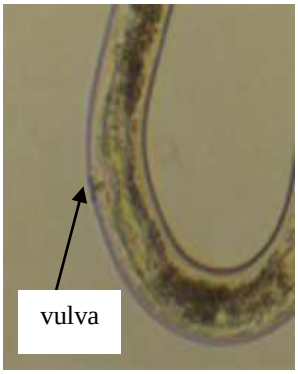
B. Female whole body

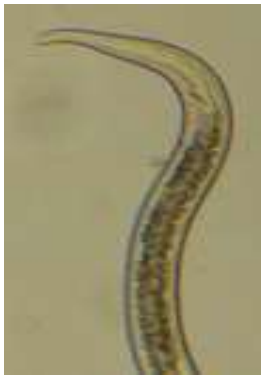
C. Male head

D. Female head



E. Male tail





F. Female vulva and tail

G. Juvenile

Plate 4. *Hirschmanniella oryzae* (Male, Female and Juvenile)

It was found out that Yarkyaw variety grown in Moegoke and Sabalsu villages surveyed. In the survey, the lowest soil and the highest roots populations of *H. oryzae* were observed in Sabalsu. In Moegoke field, soil populations of *H. oryzae* were higher than roots. This may indicate that this rice variety in both villages was infested by rice root nematodes *H. oryzae*. Manawbaykyar variety was collected from only Pyarkatat East. In soil and roots populations of *H. oryzae* in this rice field surveyed were 12/100 g soil and 35/20 g roots. Sampling was done within the first week of January when the plants were at the harvested stage. Evenly which time, *H. oryzae* was observed in survey field. Zin Thu Zar Maung *et al.*, (2013) pointed out that rice root nematode *H. oryzae* was infested in Ayeyarwady division of Myaungmya and Nyaungdone regions. Based on the present investigation, rice root nematode, *Hirschmanniella oryzae* infested in all collected varieties and villages. If the rice field infection caused by this nematode, seed quality may be decreased and yield lost.

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