PLANT-PARASITIC NEMATODES ASSOCIATED WITH MONSOON RICE IN BANMAW ENVIRONS

Khin War War[1] and Cho Nandar[2]

Abstract

A total of 17 species of plant-parasitic nematodes belongs to 15 genera, 11 families and two orders were recorded in soil and root samples at different monsoon rice fields from Banmaw environs. In two orders, the total percent of genera (73.33%) in order Tylenchida was higher than in the order Aphelenchida (26.67%). In soil samples, the highest individual population of plant-parasitic nematodes (2280/100cc soil) was recorded in after harvest sample from rice field III (RF III) and the lowest (7/100cc soil) was in second sample (S II) from rice field I (RF I). All the root samples, the highest (2008/10g root) and lowest (33/10g root) population of plant-parasitic nematodes was recorded in sixth sample (S VI) and fourth sample (S IV) respectively from RF III. Among the recorded plant-parasitic nematodes, three species (*Hirschmanniella oryzae*, *Meloidogyne graminicola*, *Aphelenchoides besseyi*) were the major rice nematodes. In all collected samples, the highest (15246 individuals) and lowest (413 individuals) individual population were observed in *H. oryzae* and *A. besseyi* respectively. The highest individual number of major rice nematodes, lowest seed weight and yield were recorded in RF III. The lowest number of nematodes, highest seed weight and yield were observed in RF I.

Keywords: monsoon rice, plant-parasitic nematodes

Introduction

Rice (*Oryza sativa* L.) is a monocotyledonous plant belonging to the grass family (Gramineae) and the genus *Oryza*. Rice is the major staple food for the 57% of the world's population and it provides approximately 23% of daily caloric intake, especially in Southeast Asia (Rehm and Espig, 1976). In many Asian countries where rice is the principal food, the importance of this crop in relation to food security and socio-economic development is evident.

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In Myanmar, rice is the national food crop and is needed for local consumption as well as for export (Dobermann and Fairhurst, 2000). There are a lot of rice diseases commonly classified into four groups which are fungus, bacteria, virus, and nematodes. Nematodes can be found in almost any type of environment and include both free-living and parasite species. Plant-parasitic nematodes typically live in soil and feed on cells in plant roots. They threaten agricultural crops throughout the world, particularly in tropical and sub-tropical regions (Oteifa, 1997). They are one of the main pathogen of rice diseases, cause annual yield loss ranging from 10 to 20% (Sharma *et al*., 2000). Nematode parasites of rice include rice white tip nematode (*Aphelenchoides besseyi*), rice stem nematode (*Ditylenlenchus angustus*), root rot nematode (*Hirschmanniella* spp.), root knot nematode (*Meloidogyne* spp.), rice cyst nematode (*Heterodera oryzae*), stunt nematode (*Tylenchorynchus* spp.), ring nematode (*Criconemoides* spp.) and lance nematode (*Hoplolaimus* spp.) (Butler, 1913; 1919). A number of genera of plant-parasitic nematodes are associated with rice in Myanmar, but only four genera of proven or potential economic importance are found. They are rice stem nematode, white tip nematode, root-knot nematode and root rot nematode (Mya Mya, 1983). In the past, emphasis had been placed on nematode disease of rice such as Ufra, white tip, root-knot in Myanmar. In the present, root-rot disease occurred in many rice growing areas of the country according to a few local reports. The present study focus on Banmaw environs because there is no information and recorded data of rice nematodes in this environs. This research seems useful to gather additional information on the incidence of nematode species on rice in Myanmar.

The objectives of the present study were to identify and record the plant-parasitic nematodes from soil, root and seed samples of rice fields, to assess the plant-parasitic nematode species composition in collected soil, root and seed samples and to compare the population of major rice plant-parasitic nematodes, seed weight and yield.

Materials and Methods

Study areas and period

Survey was carried out at different sites in Sihe (RF I), Siin (RF II) and Mophein (RF III) villages in Banmaw Township, Kachin State. This study was conducted from June 2017 to January 2018 (Plate 1 A, B, C).

Collection of soil and plant samples

Before planting, soil samples were taken with garden trowel from four corners and one center of each site and at a depth between 10 cm to 20 cm. Two weeks after planting, soil and root samples were collected fortnight interval from the same location and depth of each site. The samples were placed in an individual plastic bag with a complete label.

Extraction of nematodes from soil, root and seed

Soil samples were thoroughly mixed and 100 cc of the composite soil were used for nematode extraction by Whitehead tray method (Whitehead and Hemming, 1965). The composite soil sample was evenly spread on a cloth which was in a plastic basket that attached in a plastic tray. About 250 ml of water was carefully added down the inside edge of the tray until the soil looked wet. The tray was kept at room temperature for 24 hr. Then the basket was slowly and carefully removed and nematodes suspension from the tray was concentrated by pouring into a 250 ml beaker and the nematodes suspension was left to settle for 2 to 3 hr for nematodes to sink in the bottom. The upper layer of water was carefully decanted to remain 20 ml nematode suspension. It was thoroughly mixed by pipetting for 15 sec then 1 ml of the suspension was transferred to a counting dish. They were counted and identified under compound microscope according to Chidwood (1950), Siddiqi (1985), Hunt (2002) and Bridge (2005). Root samples were washed and cut into small pieces then mixed together. A 10 g of mixed root pieces was spread on a two layers of tissue paper in a sieve attached with a glass Petri-dish. Water from wash bottle was poured between the sieve and the Petri-dish just to cover the roots. The rest steps were the same above method.

A total of 100 seeds were bisected with a nail clipper. The split seeds and husks were placed on a two layers of tissue paper in a sieve which attached with glass Petri-dish. The rest steps were the same as mentioned above (Plate 1 D, E, F).

Results

Occurrence and Composition of Plant-Parasitic Nematodes

A total of 17 species of plant-parasitic nematodes belongs to 15 genera, 11 families, six superfamilies and two orders were recorded in soil and root samples at different monsoon rice fields from Banmaw environs (Table 1). There were *Tylenchus* sp., *Polenchus* sp., *Sakia* sp., *Boleodorus* sp., *Tylenchorhynchus* sp., *Psilenchus* sp., *Helicotylenchus dihystera*, *H. multicinctus*, *Pratylenchus* sp., *Hirschmanniella oryzae*, *Meloidogyne graminicola*, *Macroposthonia xenoplax*, *Aphelenchus* sp., *Aphelenchoides besseyi*, *A. bicaudatus*, *Ektaphelenchoides* sp., and *Bursaaphelenchus* sp. (Plate 2, 3, 4). Among them, seven species was identified to species level and the rest nematodes were classified to genera level. In two orders, the total percent of genera (73.33%) in order Tylenchida was higher than the Aphelenchida (26.67%). The highest percent of genera (26.7%) was recorded in family Tylenchidae, followed by Hoplolaimidae (13%). The lowest and similar percent of genera (6.7%) were found in each of the rest nine families (Figure. 1, 2).

Population of Plant-parasitic Nematodes in Soil and root Samples from Study Sites

The plant-parasitic nematodes were found in initial soil samples (before planting) of all different rice fields. Among them, the highest individual nematodes population (153/100cc soil) was found in rice field III (RF III) from Mophein village and the lowest (53/100cc soil) in rice field II (RF II) from Siin village. The individual population of plant-parasitic nematodes in rice field I (RF I) from Sihe was moderate (131/100cc soil). After planting, in RF I, the highest individual population of plant-parasitic

nematodes in soil sample (520/100cc soil) was observed in fifth sample, S V and the lowest population (7/100cc soil) was recorded in second sample, S II. In RF II, the highest individual population of plant-parasitic nematodes (1827/10g root) was observed in after harvest. The lowest population (53/100cc soil) was recorded in initial and second sample, S II. In RF III, the highest individual population of plant-parasitic nematodes (2208/100cc soil) was observed in after harvest sample. The lowest individual population (153/100cc soil) was recorded in initial and fourth sample, S IV. Among the all soil samples, the highest and lowest individual population of plant-parasitic nematodes was recorded in after harvest sample of RF III and second sample, S II in RF I respectively (Fig. 3). According to root samples, in RF I, the highest individual population of plant-parasitic nematodes (973/10g root) was observed in fourth sample, S IV and the lowest population (47/10g root) was recorded in second sample, S II. In RF II, the highest individual population of plant-parasitic nematodes (1173/10g root) was observed in fifth sample, S V and the lowest population (316/10g root) was recorded in first sample, S I. In RF III, the highest individual population of plant-parasitic nematodes (2008/10g root) was observed in sixth sample, S VI and the lowest individual population (33/10g root) was recorded in fourth sample, S IV. Among the all root samples, the highest and lowest individual population of plant-parasitic nematodes was recorded in sixth sample, S VI and fourth sample, S IV respectively in RF III (Fig. 4).

Population of Major Rice Nematodes in Soil and Root Samples and seed weight

Three major rice species, *Hirschmanniella oryzae* (root rot nematode), *Meloidogyne graminicola* (root knot nematode) and *Aphelenchoides besseyi* (white tip nematode) were found during study period. The highest individuals population of *H. oryzae* (7876 individuals) was observed from initial to harvested soil and root samples from RF II followed by the same population of RF I and RF III (3685 individuals/each). The highest individual population of *M. graminicola* (6825 individuals) was observed in RF III followed by RF II (1401 individuals) and RF I (554 individuals). The highest individual

population of *A. besseyi* (357 individuals) was observed in RF III, followed by RF I (42 individuals) and RF II (14 individuals) (Fig 5). In seed extraction for *A. besseyi* nematode, it was not found in all seeds samples before planting. After harvested, the seed samples from RF I was found the only one *A. besseyi* female nematode. The rest seed samples from RF II and RF III were not observed. During study period, the highest total population of major rice plant-parasitic nematode (10867 individuals) was found in RF III, followed by RF II (9291 individuals) and RF I (4281 individuals) from initial to harvested soil and root samples. In seed weight, the highest seed weight was recorded in RF I (25g/1000 seeds) followed by RF II (24g/1000 seeds) and RF III (22g/1000 seeds). The highest yield from interview survey of rice field owners was recorded in RF I (4122kg/ha) followed by RF II (3866kg/ha) and RF III (3092kg/ha). The highest individual number of major rice plant-parasitic nematodes and the lowest seed weight and yield were recorded in RF III. The lowest number of nematodes and highest seed weight and yield were observed in RF I (Figure. 6). According to the results, major rice plant-parasitic nematodes, seed weight and yield were related each other.

A. Rice field I (RE I) in Sihe village

B. Rice field II (RF II) in Siin village

C. Rice field III (RF III) in Mophein village

D. Extraction of nematode from soil

E. Extraction of nematode from root

F. Extraction of nematode from seeds

Plate1. Studied of rice fields and extraction of nematodes

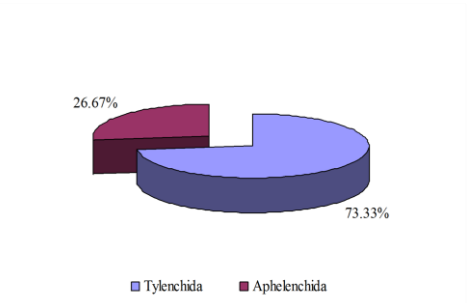


Figure 1. Percent composition of plant parasitic nematode genera in different orders

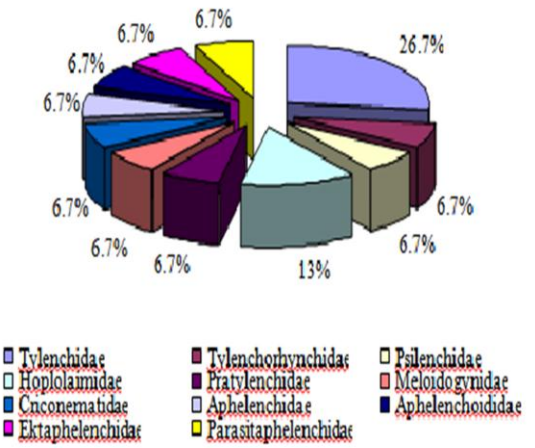


Figure 2. Percent composition of plant parasitic nematode genera in different families

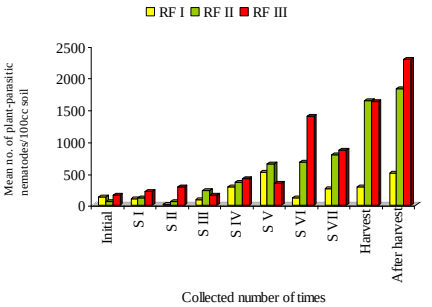


Figure 3. Plant-parasitic nematodes in all soil samples

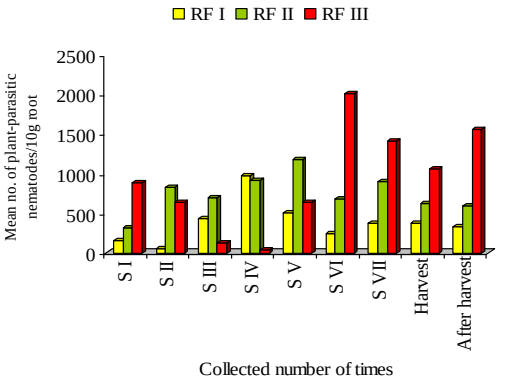


Figure 4. Plant-parasitic nematodes in all root samples

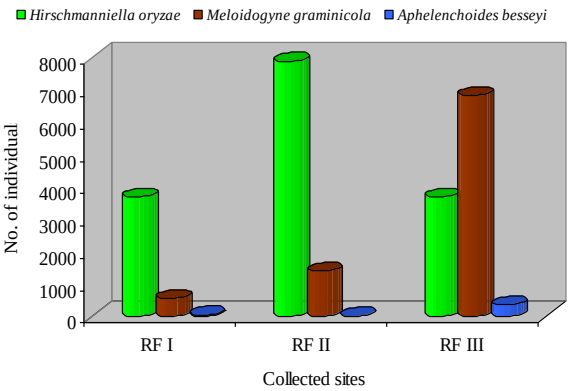


Figure 5. Individuals number of major rice nematodes population in different sites

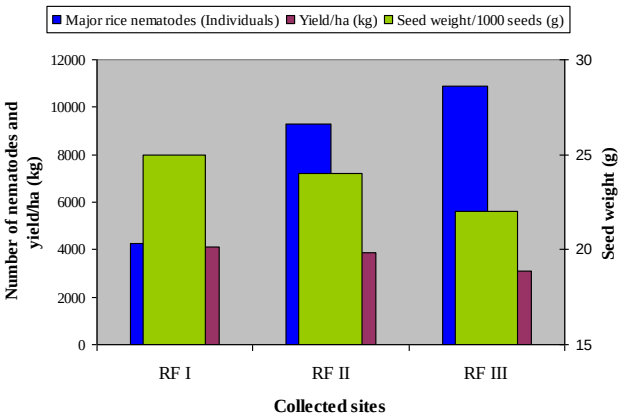


Figure 6. Major rice plant-parasitic nematodes and seed weight in different sites

A. *Tylenchus* sp. 400X

B. *Polenchus* sp. 400X

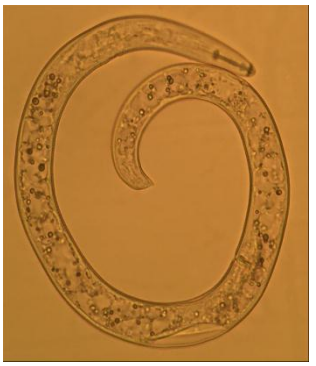
C. *Sakia* sp. 400X

D. *Boleodorous* sp. 400X

E. *Tylenchorhynchus* sp. 400X

F. *Psilenchus* sp. 400X

Plate 2. Plant-parasitic nematodes under family Tylenchidae, Tylenchorhynchidae, Psilenchidae



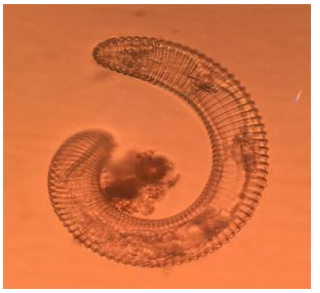
A. *Helicotylenchus dihystera* 400X

B. *H. multicintus* 400X

C. *Pratylenchus* sp. 400X

D. *Hirschmanniella oryzae* 400X

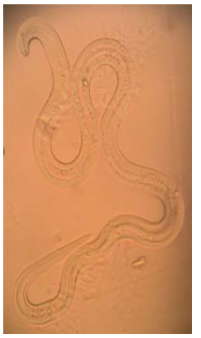
E. *Tylenchorhynchus* sp. 400X



F. *Psilenchus* sp. 400X

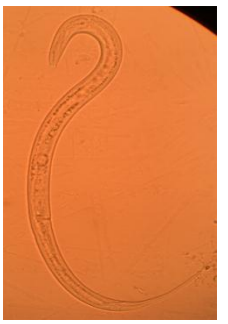
Plate 3. Plant-parasitic nematodes under family Hoplolaimidae, Pratylenchidae, Meloidogynidae, Creconematidae

A. *Aphelenchus* sp. 400X



B. *Aphelenchoides besseyi* 400X

C. *Aphelenchoides bicaudatus* 400X



D. *Ektaphelenchoides* sp. 400X

E. *Bursaphelenchus* sp. 400X

Plate 4. Plant-parasitic nematodes under family Aphelenchidae, Aphelenchoididae, Ektaphelenchidae, Parasitaphelenchidae

Table 1. Plant-parasitic nematodes genera recorded in all samples of different rice fields

Order	Superfamily	Family	Genus	Species
Tylenchida	Tylenchoidea	Tylenchidae	*Tylenchus*	*Tylenchus* sp.
			Polenchus	*Polenchus* sp.
			Sakia	*Sakia* sp.
			Boleodorus	*Boleodorus* sp.
	Dolichodoroidea	Tylenchorhynchidae	*Tylenchorhynchus*	*Tylenchorhynchus* sp.
		Psilenchidae	*Psilenchus*	*Psilenchus* sp.
	Hoplolaimoidea	Hoplolaimidae	*Helicotylenchus*	*H. dishystera*
				H. multicintus
		Pratylenchidae	*Pratylenchus*	*Pratylenchus* sp.
			Hirschmanniella	*H. oryzae*
		Meloidogynidae	*Meloidogyne*	*M. graminicola*
	Criconematoidea	Criconematidae	*Macroposthonia*	*M. xenoplax*
Aphelenchida	Aphelenchoidea	Aphelenchidae	*Aphelenchus*	*Aphelenchus* sp.
	Aphelenchoidoidea	Aphelenchoididae	*Aphelenchoides*	*A. besseyi*
				A. bicaudatus
		Ektaphelenchidae	*Ektaphelenchoides*	*Ektaphelenchoides* sp.
		Parasitaphelenchidae	*Bursaphelenchus*	*Bursaphelenchus* sp.

Discussion

In Myanmar, rice is the major agricultural crop and is grown throughout the country. The Chin thone rice variety is grown in monsoon season at Banmaw environs. The biotic and abiotic factor can reduce rice yield and seed quality. Among the biotic constraints, it contains the plant-parasitic nematodes. In the present study, a total of 17 species of plant-

parasitic nematodes belonging to 15 genera, 11 families, and two orders were recorded in soil and root samples. Previous survey carried out in 1983 in Myanmar, 28 species belonging to 22 genera of plant-parasitic nematodes from 57 host plants were reported (Mya Mya, 1983). In 2003, Po Po Than reported that 17 genera of plant-parasitic nematodes were found from 17 different crops. According to Bridge *et al*. (1978), 17 genera were found from the survey of plant-parasitic nematodes in Gambia. In U.S.A, Wehunt *et al*. (1989) reported a total of 110 species of plant-parasitic nematodes from various crops in Arkansas. The total number of nematode genera in previous records was not similar to this record. It may be due to different crops, soil types, collected time and weather condition.

During the study period, the highest (73.33%) and lowest (26.67%) percent of plant-parasitic genera was found in order Tylenchida and Aphelenchida respectively. In 2009, Aye Aye Thint, Naing Naing Oo and Aye Aye Thant reported that the high percent of genera (87%, 73.33% and 69% respectively) was found in order Tylenchida. Tylenchida was occurred in all possible habitats in soil, water and plants. Their greatest diversity of forms occurred amongst parasites of roots (Siddiqi, 1985). The finding of present work is agreed with the statements of above authors. The plant-parasitic nematodes (337 individuals/100cc soil) were found in initial sample in all study sites. A single acre of soil from arable land may contain as many as 3,000,000,000 nematodes (Hunt, 2002). The total number of nematodes is different from the statement of Hunt. It may be due to the different location, soil type and crop. Moreover the initial nematode population report was rare in previous record in Myanmar. The population of plant-parasitic nematodes in all root samples was not similar in different study sites. According to the recent results, the highest population was found in fourth sample in RF I, fifth in RF II and sixth in RF III. The differences of population may be due to the flood condition in rice fields, the growth of plant and other managements. The lowest population in all root samples was recorded in S IV at RF III. This rice field was flooded between the collected time of third and fourth samples. It is assumed that the flooding influences the nematode population. A number of

plant parasitic nematodes are associated with rice in Myanmar. There were *Ditylenchus angutus*, *Hirschmanniella oryzae*, *Meloidogyne* graminicola and *Aphelenchoides besseyi* (Aung Swe, 1997; Ohnmar Thein, 1998). During study period, *H. oryzae*, *M.* graminicola and *A. besseyi* were observed and *D. angutus* was not completely in all samples. This finding is different from the above finding. It may be due to the different study sites, variety, weather parameters, irrigation and other environmental factors.

Hirschmanniella oryzae has also been reported on rice in other Asian countries such as India, Pakistan, Bangladesh, China Korea and Japan (Bridge *et al*., 2005). Zin Thu Zar Maung (2011) conducted the collection of soil and root samples from 539 fields from 11 monsoon rice varieties in 12 regions of Myanmar. Diseased rice plants that caused by *H. oryzae* were found from rice fields of Hlaingtharyar and Htantapin Township, Yangon Region (Hla Hla Maw, 2009). In the present study, *H. oryzae* was observed in all rice soil and root samples from different rice fields. This finding is agreed with the statement of above authors. In fact, *H. oryzae* may be common in all cultivated rice fields. In the Ayeyarwady River Delta, *M. graminicola* and *H. oryzae* were found in both summer and monsoon rice growing season in delta region. Moreover no root galls were observed in monsoon rice (Pa Pa Win *et al*., 2013). During study period, both species were observed in monsoon rice in Banmaw environs and was not found the root galls. This result coincided with the above finding. According to both results, these two species may be found rice growing areas in Myanmar. The white tip nematode, *A. besseyi* was recorded in collected seeds from different rice varieties in Taikkyi, Thanutpin, Yinmarpin, Kyauktan, Thonegwa, Hlegu and Yaezin Townships. The highest individual (21/100seeds) was reported from Manawthukha rice variety in Taikkyi Township (Khin War War, 2009). In the present survey, *A. besseyi* nematode (1/100 seeds) was found in seed sample although it was found in initial soil sample from RF I and II. It may be due the different rice variety and transporting of seed from one place to another.

Among rice disease, nematode infestation can result in yield losses of up to 30 percent (Dobermann and Fairhurst, 2000). Yield loss due to the root

rot disease caused by *H. oryzae* may be as much as 50-60% (Miah and Mondal, 1988). In the screenhouse experiments, infection with *M. graminicola* caused on average a yield reduction with 31.1% in lowland rice varieties vs 44.9% in the upland rice varieties (Pa Pa Win, 2015). The numbers of *A. besseyi* nematode were found 17-34/100 seeds to reach the economic threshold level and 20-36% yield loss (Khin War War, 2009). The result of present study showed that the most important rice growing areas in Banmaw environs were all heavily infested by *H. oryzae* and *M. graminicola*. The interview survey from rice field owners, the highest yield (4122kg/ha) in Sihe was followed by Siin (3866kg/ha) and Mophein (3092kg/ha). According to this research, the highest number of major rice nematodes and lowest seed weight and yield was recorded. Moreover the lowest number of nematodes and the highest seed weight and yield were also observed.

Conclusion

A total of 17 species of plant-parasitic were recorded from different rice fields. Among them, three major rice nematodes (*H. oryzae*, *M. graminicola*, *A. besseyi*) were found in all study sites. The percent of recorded genera in order Tylenchida were higher than the Aphelenchida. Monsoon rice in Banmaw environs were heavily infested in plant-parasitic nematode. According to the nematological point of view, combination of control methods should be used to reduce nematode population. And then, finally my suggestion, seed and soil treatment should be used before planting. The ratoon and weed were burned after planting. The local farmers should be used resistant varieties, crop rotation and biological control methods

Acknowledgements

We would like to express my gratitude to Dr. Zaw Jat, Prorector, Banmaw University for his kind permission to read the research paper. We always thank to Dr Sann Sann (Professor and Head of Zoology Department) for her advice and laboratory supporting. We also thank to Dr Win Mar, Professor, Zoology Department for her advice. Finally, special thanks to rice field owners for their permission of rice samples through out the study period.

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