

Research Article

Composition of Parasitoid Wasps in Insect Pests of Rice (Rice Leaffolder, *Cnaphalocrocis Medinalis* Guenée)

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Abstract

Biological control is one aspect of Integrated Pest Management (IPM). The method focusses on natural enemies; beneficial insects are applied for biological control of agricultural insect pests and also provide an environmentally friendly approach. For the rice pests including Rice Leaf Folder (RLF), several parasitoids tend to be natural enemies that could be used to control the pest from the egg stage to pupa. This study was conducted with two varieties of rice, namely “Sen Kra Oub” and “Sro Ngea” at Battambang Province of Cambodia. Parasitoid wasps were collected from egg to pupa stages of RLF and rice yellow stem borer by rearing method, and stored in 70% of alcohol solution before sending for identification in Vietnam’s Institute of Ecology and Biological Resources (IEBR) Laboratory. The outcomes revealed that five hymenopteran parasitic wasps of three different families were found, viz. *Apanteles cypris* Nixon, *Bracon onukii* Watanabe and *Pentatermus striatus* (Szepligeti) (Braconidae), *Xanthopimpla flavolineata* Cameron (Ichneumonidae), and *Telenomus rowani* Gahan (Scelionidae) and being the primary parasitoids of rice leaffolder, *Cnaphalocrocis medinalis* Guenee; stemborer *Scirpophaga incertulas* Walker (Pyralidae) and black branded swift *Pelopidas mathias* (Hesperiidae). Parasitism was not found at the vegetative phase with Sen Kra Ob varieties but in reproductive phase. parasitism proportion was 16.66% and 28.57% to 50% in ripening phase, different from Sro Ngea variety where a proportion was found in vegetative phase 27.27%, reproductive phase at 28.57% to 40% and 28.57% to 43% in ripening phase. All the hymenopteran parasitoids were initially recorded in Cambodia, whereby all the parasitoids were potential agents for biological control for important rice insect pests. The outcome of this research suggests that the Government should invest more in the research and development for biological control in Cambodia.

Keywords

Biological Control, Parasitoid, Integrated Pest Management (IPM), Rice Leaffolder, Battambang, Cambodia

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Received: 26 June 2024; **Accepted:** 29 July 2024; **Published:** 15 August 2024



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Rice is the most important and dominant crop in Cambodian agriculture and rice-based farming systems have been the backbone of Cambodia's agriculture, with a long history [4]. It remains the dominant crop today with 80% of Cambodian people who are farmers. Cambodian farmers have a lot of experience in rice production and have developed various rice farming systems such as rainfed lowland rice, rainfed upland rice, deep-water rice, and irrigated dry season rice [8, 6] which the region experiences two difference seasons: wet season (May to October) and dry season (November to April) [9, 10].

Two major factors affecting rice production are adverse weather (floods, drought, typhoons, etc.) and pest epidemics. The major insect pests that cause significant yield losses are rice leaf folders, rice bugs, leafhoppers and plant hoppers, which cause direct damage as well as transmit viruses, stem borers, and a group of defoliator species [5]. To control rice insect pests, Cambodian farmers have traditionally applied only chemical methods which killed not only insect pests but also their natural enemies; this results in secondary pests becoming key insect pests, insecticide residues in the product, and loss of ecological balance in the rice field [3, 11]. To reduce insecticide application, it is to time think about biological control. The basis of biological control is the understanding of natural enemies of insect pests. Egg parasite wasp species are known to play an important role in suppressing insect pest populations in rice ecosystems [2] and providing an environmentally friendly and effective method of minimizing the pest damage [1], especially in places where use of broad-spectrum pesticides is avoided [7]. Therefore, a study to determine egg of parasite wasp composition is very important for building and starting biological control for rice production.

Objective

This study aims to identify the number of parasitic wasp eggs and determine the most important species for biological control. Specific objectives are to: (1) Record the proportion of parasitoids present in rice crops and population dynamics of egg parasitoid wasps; (2) provide a brief description of morphology of important egg parasitoids species; and (3) identify the most important egg parasitoid species for supporting applied biocontrol in rice plant and the number of egg parasitoids species in Battambang.

2. Methodology

2.1. Sampling Size and Proportion of Parasites

The area selection and sampling activities started during the main wet season from 13nd August until 30th November 2018 at Phnom Sampov Lech Village, Phnom Sampov commune, Banan district, Battambang province of Cambodia for two

1. Introduction

rice varieties "Sen Kra Ob" and "Sro Ngaie".

Proportion of egg-wasp parasite was recorded through collection of RLF egg mass in rice plant at seven days interval from 10 days after sowing until harvesting period. Each point sampled was 5 m²; from the first sampling to next sampling, the position was slightly adjusted to ensure sampling place does not coincide with the previous point. The collection process was conducted at five points or locations in transversal rectangular as shown in Figure 1 below.

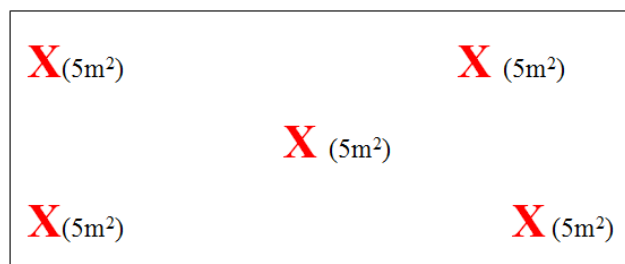


Figure 1. The area collecting egg-wasp parasite.

2.2. Data Collection

Collected eggs are kept in different test tubes with labeling by date of sampling, place of sampling, and plant host. Each sample is monitored for one week to ensure emergence of parasitoid species. Emerged parasitoids obtained from RLF eggs are preserved in 70% of alcohol solution for identification. Parasitism proportion (PR) is calculated using the equation below:

$$PR = \frac{\text{Number of parasitized eggs}}{\text{Total Collected eggs}} \times 100\%$$

2.3. Data Analysis

The sample of egg parasitoids wasp is identified in the laboratory at Meanchey University based on the parasite identification document from Institute of Ecology and Biological Resources (IEBR), Vietnam. If some species are not recorded in the documents, the samples were sent to experts in IEBR to directly identify. In rare cases, the parasitoids that cannot be identified in IEBR were sent to PESTNET group (Australia) for identification.

3. Results and Discussion

3.1. Parasitoid Composition

There are five hymenopteran species of families Bracnidae, *Scelionidae* and *Ichneumonidae* being the primary parasitoids of rice leaf folder *Cnaphalocrocis medinalis*

Guenee, *Scirpophaga incertulas* Walker (Pyralidae) and *Pelopidas mathias* (Hesperiidae) in Battambang Province, Cambodia.

Among the parasitic assemblages of rice leaf folder, *Cnaphalocrocis medinalis* and rice stem borer, *Scirpophaga incertulas*, two larval parasitoids, *Apanteles cypris* Nixon, *Bracon onukii* Watanabe, and *Pentatermus striatus* (Szepligeti) belong to the family Braconidae. All parasitoids were recorded as parasitoids of rice RLF and *Pelopidas mathias* in larval stage, but *Xanthopimla flavolineata* Cameron of the family Ichneumonidae was parasitizing the pupa stage of the leaf folder and stem borer, another parasitic wasp, *Telenomus rowani* Gahan (Scelionidae) was recorded as egg parasitoid of *Scirpophaga incertulas* Walker (Pyralidae) as shown in Table 1 below.

Table 1. Parasitoid species reared from sample of RLF in Battambang of Cambodia.

Family	Species	Host stage	Variety	
			Sro Ngeai	Sen Kro Oub
Braconidae	<i>Apanteles cypris</i> Nixon	Larvae	+	-
	<i>Bracon onukii</i> Watanabe	Larvae	+	+
	<i>Pentatermus striatus</i> (Szepligeti)	Larvae	+	-
Ichneumonidae	<i>Xanthopimla flavolineata</i> Cameron	Pupa	+	-
Scelionidae	<i>Telenomus rowani</i> Gahan	Egg	+	-

3.2. Proportion of Parasitoids in Sen Kra Oub and Sro Ngea Varieties

Based on the result (Figure 2 & Figure 3), parasitoids were not found in vegetative phase with Sen Kra Oub varieties; but at the reproductive phase, parasitoid proportion was 16.66% and 28.57% to 50% in the ripening phase, and different from Sro Ngea variety where the proportion in the vegetative phase was 27.27%, reproductive phase 28.57% to 40% and 28.57% to 43% in ripening phase.

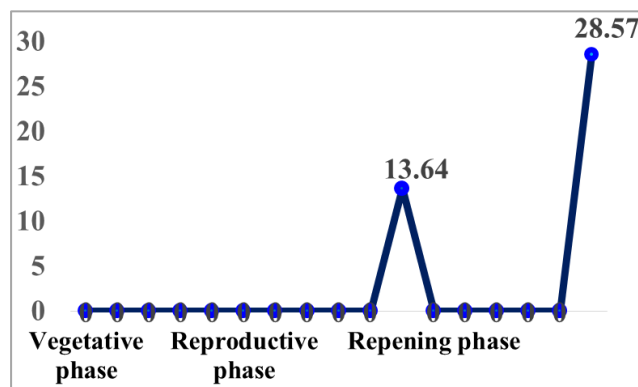


Figure 2. Proportion of parasitism on Sen Kra Oub variety.

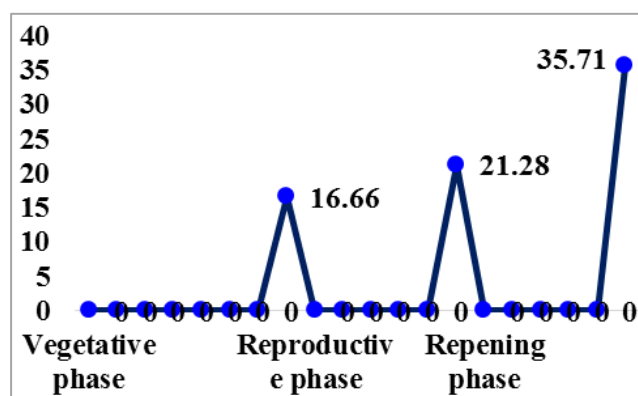


Figure 3. Proportion of parasitoid on Sro Ngea variety.

Based on the number of parasites (Figure 4 & Figure 5) in the vegetative and ripening phases, the parasitoid was not found during vegetative phase and reproductive phase. Then it occurred during ripening phase of rice at 13.63/m² (Figure 3). The highest densities of parasitoids reached its peak at 28 insect/m² before the harvesting stage. With Sen Kra Oub varieties, the parasitoid appeared only two times during the whole rice season in Battambang. The parasitoid of rice leaf folder is present in the field and this information can be used in combination with Integrated Pest Management strategies for controlling insect pests in rice fields.

It is different from Sen Kra Oub variety, where parasitoid proportion was higher proportion than the Sro Ngea variety. It was found three times during the reproductive stage, ripening stage, and at the end of harvesting stage at 16.67%, 21.27%, and 35.71%; respectively. The reason for this could be that Sen Kra Oub is more susceptible to damage from rice leaf folder than in Sro Ngea; and the farmers used more insecticides to control rice leaf folder. This may have resulted in insecticides killing the parasitoid from an early stage.

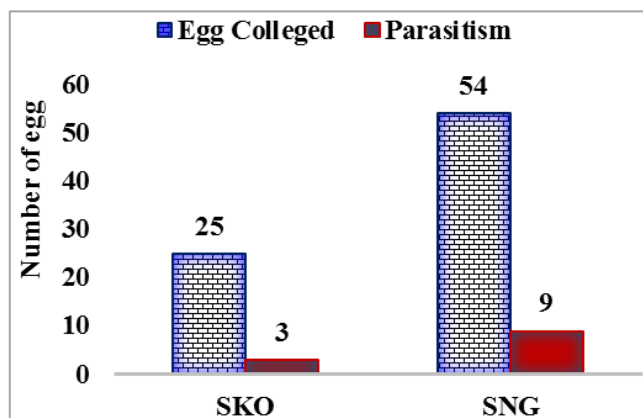


Figure 4. Number of egg parasites in the vegetative phase.

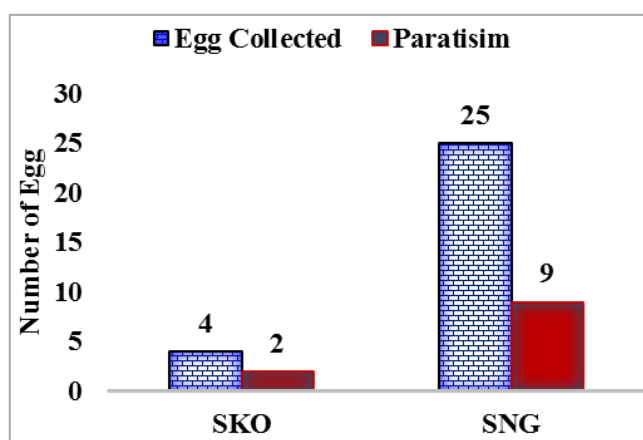


Figure 5. Number of egg parasites in the ripening phase.

3.3. Morphology of Parasitoid

Apanteles cypris Nixon (Figure 6): Differs essentially from *apacus* in having tergite shorter, wider and not or have narrowed behind; the median field of tergite is, in consequence, more transverse. Disc of scutellum distinctly punctate but the punctures more obvious on anterior half. Tergite with a distinctly longitudinal channel, margined along each side. Ovipositor shape about three quarters as long as the hind tibia.

Xanthopimla flavolineata Cameron (Figure 7): Head. Clypeus weakly convex; face flat; eyes converged anterior; antennae with 36 flagellar segments. Mesosoma. Notaulus short, shallow; mesoscutal crest almost present; scutellum weakly convex; anterior lower corner of pronotum with rounded angle; subtegular ridge rounded; postpectal carina simple; tubercle absent; pleural area not divided; costula complete; apical transverse carina complete; areola completely surrounded by carina; upper lateral longitudinal carina on

propodeum present more than 0.5x length of outer margin of first lateral area on propodeum areola; propodeum with areola separate from second lateral area and petiolar are. Wings. Areolet of forewings closed; second recurrent vein before or in the middle of areolet; nervulus opposite with basal vein; distal hamuli 6. Legs. Apical bristles 5-6; Preapical bristles 5; inner hairs of hind tarsal claws curved, wide and blackened tip. Metasoma. Dorsolateral carina partly present, long; ovipositor tip straight; upper valve equal with lower valve; upper valve without tooth; lower valve 7 tooth. Color pattern. Mesoscutum; propodeum; coxa belakage; femur and hind tibia are yellow brownish; forewing weakly infuscate; stigma brownish; tergite 1 to 8 yellowish with brownish rhombic area. Variation. Apical bristles 4-6.

Bracon onukii Watanabe (Figure 8): Colour pattern. Reddish yellow; eyes, tips of mandibles, claws, and ovipositor-sheaths black; three lobes of mesonotum, propodeum, and the first three abdominal tergites often with black markings. Antennae yellowish brown, darken towards the apex. Wings hyaline, stigma and vein yellow. Head. Smooth and shining, antennae filiform, shorter than the body, the scape cylindrical. Thorax smooth and shining; parapsidal furrows of the mesonotum deep, reaching to the apex; mesopleural foveae broad. Second abscissa of the radius 2.5 times longer than the first; the first intercubital nervure oblique, the second vertical; 2nd cubitus 1.5 times longer than the 2nd abscissa of the radius. Legs. Normal. Propodeum almost smooth and shining, with a median longitudinal carina from apex to the middle, crossed by some transverse carinae. Abdomen rugosely reticulate dull; 1st tergite margined laterally, the median raised area round; 2nd tergite longer than the 3rd, with a fine short median carina at base; suture between the 2nd and the 3rd being broad and deep, almost straight. Ovipositor as long as half the length of abdomen 1mm. Length 3.5 mm.

Telenomus rowani Gahan (Figure 9): It is very small and slender species. metasoma long and slender, distinctly concave in dried specimens; antennae slender, rather long, four apical segments slightly enlarged to form a club, but the proximal segment not distinctly large; four basal funicle segment very short, round, each segment shorter than pedicel which is also short (a little longer than broad); head rounded, rather small, scarcely broader than thorax; length 0.8 mm.

Pentatermus striatus (Szepligeti) (Figure 10): Body light reddish brown or brownish yellow. Abdomen wider. Dorsal-carinae of 1st tergite parallel-sided or convergent in apical 2/3. 2nd tergite 1.7 – 1.8 times as long as 3rd tergite. Propodeum smooth or punctulate basally. Ovipositor sheath 0.17 – 0.2 times as long as fore wing.



Figure 6. *Apanteles cypris* (female).



Figure 10. *Pentatermus striatus* (Szepligeti).



Figure 7. *Xanthopimpla flavolineata* (female).

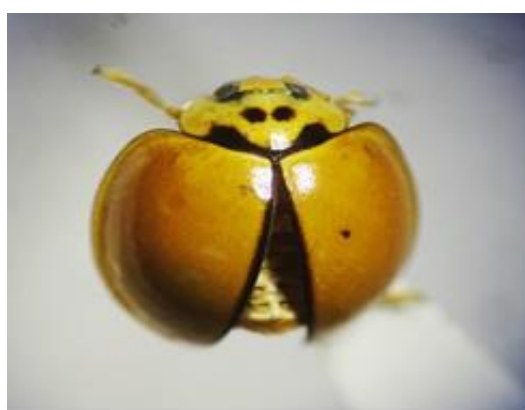


Figure 11. *Micraspis* spp.



Figure 8. *Telenomus rowani* Gahan.



Figure 12. *Oxyopes lineatipes* (C.L. Koch).



Figure 9. *Bracon onukii* (female).

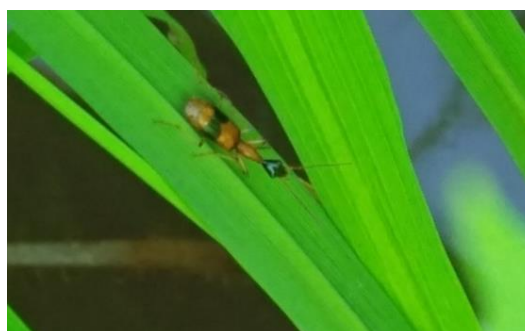


Figure 13. *Ophionea* spp.

Table 2. *Parasitic Species and Distribution.*

Parasitic	Host	Parasitism	Distribution
Family: Braconidae			
<i>Apanteles cypris</i> Nixon	<i>Cnaphalocrocis medinalis</i>	Larvae	Eastern Palearctic & Oriental: Bangladesh, Philippines, India, China, Indonesia, Japan, Nepal, Malaysia, Pakistan, Singapore, Sri Lanka and Vietnam.
<i>Bracon onukii</i> Watanabe	<i>Cnaphalocrocis medinalis</i>	Larvae	Eastern Palearctic & Oriental: China, Japan, Korea, and Vietnam.
<i>Pentatermus striatus</i> (Szepligeti)	<i>Cnaphalocrocis medinalis</i>	Larvae	Eastern Palearctic, Ethiopian, And Oriental: China, India; Indonesia, Japan, Malaysia, Niger, Nigeria, Oman, Somalia, South Africa, Vietnam.
<i>Dolochogenidea agilis</i> Ashmead	<i>Pelopidas mathias</i>	Larvae	Oriental: Philippines, Indonesia, India and Vietnam.
<i>Tropobracon luteus</i> Cameron	<i>Scirpophaga incertulas</i> <i>Chilo suppressalis</i> and <i>Sesamia inferens</i>	Larvae	Oriental: Indonesia, Malaysia, Pakistan, Philippines, Thailand, Vietnam, Bangladesh, China, India, and Sri Lanka.
Family: Scelionidae <i>Telenomus rowani</i> Gahan	<i>Scirpophaga incertulas</i>	Egg	Japan, Korea, Formosa, China, Indo-China, Philippines, Siam and Java.
Family: Ichneumonidae			
<i>Xanthopimpla flavolineata</i> Cameron	<i>Cnaphalocrocis medinalis</i>	Larvae	Australasian, Oceanic, Oriental: Australia, Indonesia, Philippines, Japan, Laos, Malaysia, Nepal, Pakistan, Papua New Guinea; Sri Lanka, Bangladesh, Vietnam.
<i>Casinarina colacae</i> Sona	<i>Parnara guttata</i> ; <i>Pelopidas mathias</i>	Larvae	Eastern Palaearctic & Oriental: China.

During the collection, the other natural enemies on rice insect were found (shown in Table 3): *Micraspis* spp. (Figure 11), *Oxyopes lineatipes* (C.L. Koch) (Figure 12). *Ophionea* spp. (Figure 13), *Coccinella* spp (Figure 14), *Tetragnatha* spp. (Figure 15) and *Agriocnemis femina* (Brauer) (Figure 16) and All the hymenopteran parasitoids in this study were recorded for the first time in Cambodia. Additionally, all the parasitoids revealed were the potential agents for biological control for the important rice insect pests.

**Figure 14.** *Coccinella* spp.**Figure 15.** *Tetragnatha* spp.**Figure 16.** *Agriocnemis femina*.

Table 3. *The Other Natural Enemies in Battambang fields.*

Species	Host Attract	Description
Micraspis sp.	planthopper	Oval and brightly colored in shades of red. Lady beetles are active during the day in the upper half of the rice canopy in dryland and wetland habitats.
Ophionea spp.	leaf folder larvae.	Active hard-bodied insects. Both the shiny black larvae and reddish-brown adults
Coccinella spp.	planthopper nymph.	Are black-spotted lady beetles that catch slow-moving prey. Adults are quick to fall off plants or fly when disturbed. The males can be recognized by the enlarged jaws
Tetragnatha spp.	leafhopper prey	Long legs and bodies and are commonly seen lying outstretched along a rice leaf. The eggs are laid in a mass covered in cottony silk.
Agriocnemis femina (Brauer)	leaf folder moth.	The narrow-winged damselflies are weak fliers compared with their dragonfly cousins. Males are more colorful than females. Male has a blue-green abdominal tip and sides of the thorax while the female has a greenish body.
Oxyopes lineatipes (C.L. Koch)	Planthopper, Leafhopper.	The hunters and build no webs, It is has two reddish brown and two white stripes running along the abdomen. The female guards its cocoon-like egg mass laid on foliage.

4. Conclusions

During early wet season and wet season of rice in the North West Cambodia 2018, five species of parasitoids of rice leaf folder found in the rice field; one species of parasite on egg, one species of parasite on pupae, and three species of parasites on larva. The population dynamics of the parasitoid were recorded in two rice varieties: “Sen Kra Oub” and “Sro Ngea”. The proportion of parasitoids in Sen Kra Oub was higher than in Sro Ngea varieties. This data are very useful for building an integrated pest management program and applied as a foundation for establishing biological control of rice leaf folder.

All the hymenopteran parasitoids were initially recorded in Cambodia whereby all the parasitoids presented the potential agents for biological control for important rice insect pests. The outcome of this research suggests that the Government should invest more in the research and development of biological control in Cambodia. Another suggestion is for a survey in the dry season for natural enemies and promoting integrated pest management (IPM) in Cambodia to enhance the sustainability of the environment and pesticide reduction to minimize the negative impacts on rice farmers.

Abbreviations

IEBR	Institute of Ecology and Biological Resources
IPM	Integrated Pest Management
PR	Parasitism Proportion
SKO	Sen Kra Oub Variety of Rice
SNG	Sro Ngea Variety of Rice
RLF	Rice Leaf Folder
%	Percentage

Author Contributions

Khem Sokheng: Conceptualization, Investigation, Methodology, Writing – original draft

Siek Darith: Data curation, Formal Analysis, Funding acquisition, Software, Visualization, Writing – original draft, Writing – review & editing

Try Yorn: Conceptualization, Formal Analysis, Funding acquisition, Methodology, Project administration, Software, Supervision, Visualization, Writing – review & editing

Heng Muy Kim: Funding acquisition, Resources, Software

Leng Channy: Data curation, Investigation, Resources

Conflicts of Interest

The authors declare no conflicts of interest.

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