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RESEARCH ARTICLE

Assessment of the egg parasitoid guild of rice leaf folder, *Cnaphalocrocis medinalis* (Guenee): a search for biocontrol agents

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Abstract: Biological control of rice leaf folder (RLF), *Cnaphlocrocis medinalis* by augmentative release of egg parasitoids has been suggested. This study was conducted to assess the egg parasitoid guild associated with the RLF and to determine their parasitism levels under local conditions. A survey was conducted in three districts at 14 sites using 16 fields where rice was cultivated under conventional, pesticide free and organic farming practices. RLF eggs were collected and incubated at room temperature with 60 % RH under natural daylight in the laboratory. The emerging parasitoids were identified and their parasitism levels were calculated.

Four egg parasitoid species: *Troichogrammatoidea bactrae, Troichogrammatoidea nana, Trichogramma* sp. and *Lithromeromyia* sp. (Hymenoptera: Trichogrammatidae) were found parasitising RLF eggs. These species collectively parasitised 17 % of RLF eggs and the level of parasitism significantly varied between 0 and 57 % among sampling sites. Mean parasitism in conventional and pesticide free fields were 1.62 ± 0.81 % and 24.39 ± 16.8 %, respectively, which was significantly different. *T. bactrae* was the most prevalent parasitoid species, which was found in nine sites, while *T. nana* and *Lithromeromyia* sp. were found only in one site. *Trichogramma* sp. was found in four sites. Mean parasitism caused by *T. bactrae, T. nana, Trichogramma* sp. and *Lithromeromyia* sp. were 8.2 ± 5.7 %, 15.2 %, 3.7 ± 1.3 % and 2.5 %, respectively. The parasitoids did not affect beneficial arthropods such as spiders and coccinellids. The results suggest that *T. bactrae* is a potential egg parasitoid of RLF to manage RLF populations in local rice fields through augmentative release.

Keywords: *Cnaphalocrocis medinalis*, egg parasitoids, Sri Lanka, Trichogrammatidae, *Trichogrammatoidea bactrae.*

INTRODUCTION

Rice leaf folder (RLF), *Cnaphalocrocis medinalis* (Guenee) (Lepidoptera, Pyralidae) is considered as one of the major defoliating insects damaging the rice crop (DOA, 2006) in all rice growing areas of Sri Lanka (Wickramasinghe, 1980). RLF adults lay eggs either singly or in pairs on the leaf blades of the rice plant and the incubation period is four days (Ranasinghe, 1992). There are five larval instars in the life history of the RLF and the larvae fold the edges of a leaf and construct a leaf roll. They live inside the rolled leaf feeding on the mesophyll cells of the leaf blades. The damage caused by the RLF larvae affects the growth of the plant leading to subsequent microbial infections (Pathak & Khan, 1994). Yield loss associated with RLF damage has been estimated as 10 % depending on the level and time of attack (Mikuriya *et al.,* 1988). However, some studies have suggested that the yield reduction associated with RLF infestation is minimal (Asghar *et al.,* 2009; Perera & Hemachandra, 2013). In Sri Lanka, 10 % of rice crop is affected annually by the RLF (Nugaliyadde *et al.,* 2001).

Several methods have been established to manage the RLF (Pathak & Khan, 1994). Chemical control is the conventional and commonly used method for RLF control. The Department of Agriculture of Sri Lanka has recommended several insecticides for managing RLF

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such as Fipronil 50 g/L SC, Tebufenozide 200 g/L SC, Methoxyfenozide 240 g/L SC *etc*. (DOA, 2010). However, negative impacts of insecticide application such as the destruction of beneficial natural enemies, environmental pollution, health hazards through occupational exposure, chronic ailments and increasing cost of paddy production are well established (Koul & Cuperus, 2007). Hence, there is a need to find alternate methods of RLF control such as host plant resistance (HPR) or biological control. The method of HPR has been tried (Henrichs *et al.,* 1985), but only a few rice varieties are available with adequate resistance against RLF.

Biological control of insect pests involves the use of entomopathogenic microbes such as *Bacillus* spp., *Beveria bassiana* and biocontrol agents such as parasitoids. Entomopathogenic microbes can be mass produced and sold as biopesticides (Sheng *et al.,* 2002). However, the use of these biopesticides in tropical areas is still problematic due to strong sunlight and high temperatures. The impact of larval and pupal parasitoids on RLF is not adequate to control the pest, and other control strategies are needed because these parasitoids attack only the later stages of the RLF, and by that time the damage to the crop is extensive. Therefore, the use of larval and pupal parasitoids has not become popular (De Kraker, 1996).

The approach of releasing egg parasitoids has been successful and appears to be one of the potential strategies to manage RLF populations (Kumar & Khan, 2005). This approach requires the identification of locally available egg parasitoids. In some countries, the egg parasitoid guild of RLF has been characterised (De Kraker *et al.,* 1999). In Sri Lanka the larval and pupal parasitoids have been identified (Rajapakse, 1990) but there are no records of egg parasitoids of RLF. *Trichogramma chilonis, Trichogramma closterae, Trichogramma confusum, Trichogramma dendrolimi, Trichogramma japonicum, Trichogramma leucaniae, Trichogramma ostriniae, Trichogrammatoidea nana* and *Trichogramma* spp. have been recorded as egg parasitoids of RLF in India, The Philippines, Malaysia and China (Vreden & Ahmadzabidi, 1986; Gurr *et al.,* 2012).

This study was conducted with the following objectives. The assessment of the locally present egg parasitoid guild of RLF and assessment of the degree of parasitism by each species at different locations under two management practices: the conventional (with the use of pesticides) and pesticide free cultivation systems.

METHODOLOGY

This study was conducted during December 2011 – August 2013. The study had three major components: (a) field collection of parasitoid eggs, (b) laboratory rearing of collected eggs and (c) the identification of egg parasitoid adults.

Field collection of parasitoid eggs

Field collections were conducted in selected rice growing areas in Kandy, Kurunegala and Anuradhapura Districts. The selected fields had been cultivated under two cultivation systems: conventional and pesticide free. The selection of rice fields was done based on the presence of RLF infestation, convenient access for regular sampling and the history of pesticide usage.

Sixteen rice fields in 14 sites from 3 districts were selected for the study (Table 1). The number of samples collected from these fields varied from 1 – 30 depending on the level of infestation and the logistics associated with visiting the fields. Some of the fields were visited at weekly intervals to collect RLF eggs.

During sampling, leaf blades and leaf sheaths of rice plants were examined using a hand lens. If RLF eggs were found, they were collected along with the leaf blade into a clean and clear plastic vial (5.5 cm height and 2.5 cm diameter). The plastic vials with eggs were stored in a cool box and transported on the same day to the laboratory of the Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya.

During the collection, eggs of other beneficial insects like coccinellids, spiders and *Andrallus spinidens* were also collected using the above procedure.

Laboratory rearing of egg parasitoids

In the laboratory, the collected eggs were examined under the dissecting microscope (Meiji, Japan 1-7 × 10) for un-hatched eggs or egg masses and they were placed individually in clear plastic vials for incubation. In the case of egg masses where the eggs could not be separated, each egg mass was placed in clear plastic vials separately. Each vial was labelled with a code that was tagged with all collection details. The eggs were incubated at room temperature (26 ± 2 ^{0}C) and 60 % RH under natural daylight in the laboratory. Vials were examined daily for emergence of host larvae or egg parasitoid adults. Upon emergence of adult parasitoids, they were preserved in 70 % ethanol in plastic vials separately with necessary information until further laboratory examination for identification. The identity of each and every parasitoid adult was determined and recorded.

Identification of egg parasitoids

Adult egg parasitoids were identified according to the protocol described by Noyes (2013). The male parasitoids were separated by the characteristics of the antennae. They were carefully mounted on cards and using external morphology, the different morphological species were identified.

Card mounted specimens were used for the preparation of glass slides by adopting the procedure described by Platner *et al.* (1999). The wings were separated and mounted in Canada balsam on glass slides. The remaining parts of the body were separated and boiled in 10 % KOH for 5 min, washed and processed in ethanol series and clove oil. The dehydrated

Table 1: Sampling sites where rice leaf folder eggs were collected for assessment of egg parasitoid guild during 2011 - 2013

Sampling sites	Latitude and longitude	Type of field	Sampling period	Surrounding of the sampling site
Karangoda	6^{0}67´N 80^{0}36´E	C	23.12.2011 - 03.02.2012	Field is surrounded by large perennial plants, isolated field
Dellabada	6^{0}65´N 80^{0}37´E	C	23.12.2011 - 03.02.2012	One side of the field borders a main road, and other three sides were bordered by paddy fields
Hindagala (3 sites)	7^{0}23´N 80^{0}60´E	C	16.01.2012 - 26.12.2012	One side of the field borders a cassava plantation and other three sides were bordered by paddy fields
Gelioya	7^{0}21´N 80^{0}59´E	C	03.01.2012 - 10.02.2012	One side of the field borders a main road, and other three sides were bordered by paddy fields
Ihalawela	7^{0}29´N 80^{0}72´E	PF	30.12.2011 - 14.02.2012	Surrounded on all sides by paddy fields that were managed by conventional methods
Bathalagoda	7^{0}48´N 80^{0}36´E	O	27.01.2012	One side of field bordered by a conventional rice field, and the other three sides by organic fields.
Bathalagoda	7^{0}48´N 80^{0}36´E	C	27.01.2012 - 09.08.2012	One side of the field bordered by a narrow road, and other three sides bordered by conventional paddy fields.
Mahailluppallama	8^{0}06´N 80^{0}53´E	C	28.02.2012 - 18.03.2013	Surrounded by paddy fields managed using conventional methods
Pallealudeniya	7^{0}12´N 80^{0}56´E	C	28.06.2012 - 30.07.2012	Surrounded by paddy fields managed using conventional methods
Megodakalugamuwa	7^{0}18´N 80^{0}71´E	C	07.06.2012 - 24.07.2012	Surrounded by paddy fields managed using conventional methods
Penideniya	7^{0}25´N 80^{0}58´E	PF	07.09.2012 - 11.10.2012	Pesticide free field surrounded by large plants and vegetable plants
Mawathagama	7^{0}43´N 80^{0}44´E	C	24.01.2013	Surrounded by paddy fields managed using conventional methods
Ganegoda	7^{0}20´N 80^{0}60´E	C	06.02.2013 - 21.03.2013	Surrounded by paddy fields managed using conventional methods
Wathurakumbura	7^{0}29´N 80^{0}54´E	PF	08.01.2013 - 12.08.2013	Pesticide free paddy field surrounded by banana trees and other large trees

C: Conventional fields; PF: Pesticide free fields; O: Organic field specimens were mounted in thin balsam on a glass slide under the dissecting microscope. The antennae, head capsule, wings, body and the genitalia were mounted separately in balsam and covered with 8 mm diameter cover glasses.

The prepared slides were kept at 35 °C in an oven for 10 days until the slides were well dried. The slides were examined under a compound light microscope (40 × 10) and the genitalia structure was compared with the sketches given in *Trichogramma* identification keys and the original descriptions, sketches given when first describing the species (Nagaraja, 1978). Confirmation of the species identity was made by Dr Andrew Polazeik, Natural History Museum, London, United Kingdom followed by molecular identification at the University of California, USA.

The degree of parasitism was estimated as the number of parasitoid adults that emerged divided by the total number of host eggs multiplied by 100 (Van Driesche, 1983). The data were analysed using Chi-square test in SYSTAT (version 11).

RESULTS AND DISCUSSION

A total of 13,733 eggs were collected from 161 samples in 16 fields. The number of eggs collected from each field significantly varied among the sampling fields ($X^2 = 11997.5$, df = 15, $p < 0.001$). The highest number of eggs was collected from the field at Penideniya, which had been maintained as a pesticide free field (Table 2). The lowest number of eggs was found in the Mawathagama field, which had been maintained using conventional methods. Generally, slightly more RLF eggs (54.5 %) were collected from conventionally managed fields compared to the pesticide free fields.

Four different species of egg parasitoids were identified. They were all Trichogrammatids (Hymenoptera, Chalcidoidea). The species were *Trichogrammatoidea bactrae* Nagaraja, *Trichogrammatoidea nana* Zehntner, *Lathromeromyia* sp. and *Trichogramma* sp. The identities of the first two species were confirmed by Dr A. Polazek, Natural History Museum, London, United Kingdom. According to the Universal Chalcidoidea database and the other records available, this is the first record of *T. bactrae* and *T. nana* in Sri Lanka. *Lathromeromyia* sp. was found only from the Mahailluppallama rice field located in the Dry Zone. This species had been found to attack hemipteran eggs in rice fields as well as in other fields (Yousuf & Shafee, 1987).

According to previous studies, *Trichogramma chilonis, T. closterae, T. confusum, T. dendrolimi, T. japonicum, T. leucaniae, T. ostriniae* and *Trichogrammatoidea nana* have been reported as egg parasitoids of RLF (Vreden & Ahmadzabidi, 1986; Gurr *et al.,* 2012). Of these species, *Trichogramma chilonis* and *Trichogramma japonicum* (Bentur *et al.,* 1994; Kumar & Khan, 2005) have been found in Indian rice fields, which are similar to Sri Lankan rice fields.

The numbers of RLF eggs collected from conventional rice fields and pesticide free fields were significantly different ($X^2 = 22.17$, df = 1, $p < 0.001$) and were associated with the level of RLF infestation, the number of samples and sampling durations. The total number of eggs collected from pesticide free fields was slightly lower than the number collected from conventional fields.

Out of the eggs collected in the fields, 2338 eggs were parasitised with the mean parasitism of 17.02 %. This finding agrees with a result of a similar study by De Kraker *et al.* (1999), in irrigated rice in the Laguna Province, the Philippines. The same study reported egg parasitism of *C. medinalis* by *Trichogramma* to be 0 – 27 %.

The total number of parasitised and unparasitised eggs significantly varied among sampling sites ($X^2 = 5585.47$, df = 15, $p < 0.001$). The highest level of parasitism of RLF eggs by all egg parasitoid species was recorded in the Penideniya field, which was 57.29 %. Depending on the sampling sites, total parasitism of RLF eggs varied between 0 and 57 % (Table 2). Except in the Penideniya field, parasitism in other fields varied between 0 and 14 %. The variation of the level of parasitism among the sites is possible as the level of parasitism depends on site characteristics such as the history of pesticide application, characteristics of surrounding fields, scale of farming in the area, rice variety, differences in the population size of the parasitoids, differences in the ability of parasitoids to find and parasitise their hosts, and differences in landscape structure *etc.* (Hunter, 2002). Geographical feature variation among the sites also has an influence on the variation of parasitism (Hunter, 2002). Exceptionally high level of parasitism in the Penideniya field might be associated with flowering vegetation and high number of plant species in the surrounding area. Parasitism of RLF eggs by *T. nana* (15 %) has contributed largely to the total parasitism, which was absent in other sampling sites. Stephens *et al*. (1998) have found higher parasitism rates in crops with nearby flowering vegetation. Weeds are beneficial to parasitoids as alternate hosts, complementary hosts, as a food source (pollen and nectar) or as resting sites for adults (Norris & Kogan, 2000).

The level of parasitism of RLF eggs by all egg parasitoid species in conventional fields, pesticide free fields and organic field were 1.62 ± 0.81 %, 24.39 ± 16.8 % and 0.68 % respectively, which was significantly different ($X^2 = 3212.57$, df = 2, $p < 0.001$). It was expected to have a high level of parasitism in organically managed fields but it was not found to be so.

The organically managed field was under organic practices only for a short period of time (two years) and one side was bordered by a conventional paddy field. These factors might have influenced to produce a low level of parasitism in the organic field. The level of parasitism of RLF eggs by all egg parasitoids significantly varied between pesticide free and conventionally managed fields ($X^2 = 3104$, df = 1, $p < 0.001$). This reflects the impact of pesticides on the existence of egg parasitoids in the field, which agrees with the data from many similar studies (Buchori *et al.,* 2010; Gnanakumar *et al.,* 2012). Parasitoids usually have a very low survival if the environment is contaminated with pesticide residues (Norris & Kogan, 2000).

Table 2: Parasitism of rice leaf folder eggs by different species of egg parasitoids in 16 different rice fields.

Location	No. of samples	No. of RLF eggs collected	No. of emerged parasitoids	Parasitism (%)				
				T. bactrae	*T. nana*	*Trichogramma* sp.	*Lathromeromyia* sp.	Total parasitism (%)
Karangoda	6	555	0	0	0	0	0	0
Dellabada	6	451	0	0	0	0	0	0
Hindagala-A	14	707	16	2.26	0	0	0	2.26
Hindagala-B	16	997	13	1.30	0	0	0	1.30
Hindagala-C	18	913	2	0.22	0	0	0	0.22
Gelioya	6	382	0	0	0	0	0	0
Ihalawela	7	852	16	1.88	0	0	0	1.88
Bathalagoda	1	293	2	0	0	0.68	0	0.68
Bathalagoda	14	1175	0	0	0	0	0	0
Mahailluppallama	3	376	26	2.23	0	2.50	2.50	6.91
Pallealudeniya	5	346	0	0	0	0	0	0
Megodakalugamuwa	30	1151	9	0.78	0	0	0	0.78
Penideniya	16	3526	2020	53.76	15.15	0	0	57.29
Mawathagama	1	147	0	0	0	0	0	0
Ganegoda	4	289	23	2.91	0	5.33	0	7.96
Wathurakumbura	14	1573	220	8.76	0	6.23	0	13.99

Trichogrammatoidea bactrae was the most prevalent parasitoid species in all sampling sites. However, the parasitism level of RLF eggs by *T. bactrae* varied across sampling locations ($X^2 = 2873$, df = 15, $p < 0.001$). The highest level of parasitism by *T. bactrae* was 53.76 % from the Penideniya field where exceptionally high level of parasitism was found. But in the other fields, it varied between 0 and 9 %. It is possible that one parasitoid species dominates a parasitoid guild due to its ability to better survive in the environment, a comparatively high efficiency in terms of host search behaviour and efficient parasitism (Hassell & Waage, 1984; Getz & Mills, 1996). The level of parasitism by *T. bactrae* significantly varied ($X^2 = 1695.19$, df = 1, $p < 0.001$) between pesticide free and conventional fields (Table 2) indicating better survival of *T. bactrae* in pesticide free fields, which agrees with a well documented study (Hafez *et al.,* 1996). Considering the prevalence of *T. bactrae* in all fields where parasitism was reported, and the high survival in both types of fields, it is suggested that *T. bactrae* is a potential candidate for biological control of RLF.

Trichogrammatoidea nana was found only in the Penideniya rice field where the highest level (15 %) of parasitism of RLF eggs was reported. It appears that *T. nana* is not a widely distributed species. The host range of *T. nana* includes mainly lepidopteron insects such as *Hypsiphyla* sp. (Sands & Murphy, 2001).

Trichogrammatoidea nana is capable of parasitising the eggs of *Agryroploce schistocena, Bactra venosana, Chilo sacchariphagus, C. infuscatella, Corcyra cephalonica, Diatraea obliqua, Ephestia cautella, Eublemma amabilis, Hapalia macheralis, Holcocera* *pulverea, Hyblea puera, Hypsipyla robusta, Chickrassia* sp., *Swietenia macrophylla, Sylepta derogata, Thosea assigna* and *Tarucas theophrastus* (Kazmi & Chauhan, 2003). Considering the host range of *T. nana,* it is possible that the RLF might not be a preferred host or the rice ecosystem might not be a preferred habitat.

Trichogramma spp. was found in four sampling sites (Table 2) in Kandy, Kurunegala and Anuradhapura Districts and the level of parasitism of RLF eggs varied between 0.68 and 6.2 %. The level of parasitism significantly varied among the four sampling sites ($X^2 = 78.4$, df = 3, $p < 0.001$) (Table 2). These four sampling sites had been managed under conventional farming, pesticide free and organic practices and the level of parasitism significantly varied among the three management methods ($X^2 = 52.1$, df = 2, $p < 0.001$); the parasitism levels were 1.3, 5.2 and 0.7 %, respectively. However, it should be noted that the parasitism in the organic field is based on one sample with 293 RLF eggs.

Table 3: Parasitism of eggs of natural enemies of rice pests in sixteen different rice fields in three geographical zones

Location	No. of samples	Host species	No. of host eggs collected	No. of emerged parasitoids
Karangoda	6	Spider eggs	31	0
Dellabada	6	Spider eggs	23	0
Hindagala-A	14	Spider eggs	31	0
Hindagala-B	16	Spider eggs	48	12
Hindagala-C	18	Spider eggs	37	0
Gelioya	6	Spider eggs	21	0
Ihalawela	7	Spider eggs	49	0
Bathalagoda	15	Spider eggs	32	0
Mahailluppallama	3	Spider eggs	28	37
		Coccinellid sp.	15	0
		Andrallus spinidens (F.)	40	5
Pallealudeniya	5	Spider eggs	8	0
Megodakalugamuwa	30	Spider eggs	11	0
Penideniya	16	Spider eggs	104	31
		Coccinelid sp.	20	0
Mawathagama	1	Spider eggs	3	0
Ganegoda	4	Spider eggs	28	4
Wathurakumbura	14	Spider eggs	105	23
		Coccinelid sp.	15	0

Lathromeromyia sp. was found in the Mahailluppallama rice field parasitising RLF eggs, which was based on two samples with nine parasitised eggs out of 362 RLF eggs. *Lathromeromyia* sp. generally parasitise the hemipteran eggs, but not lepidopteran eggs.

The non target effect of egg parasitoids was assessed by taking samples of eggs of naturally prevailing natural enemies (Table 3). Eggs of coccinellid species, predatory bug (*Andrallus spinidens*) and spider species are commonly found in the rice ecosystem. The egg masses of natural enemies had been parasitised in collections made in five locations (Table 3); however, the emerged parasitoids were not *Trichogramma* or *Trichogrammatoidea* species. This suggest that augmentation and release of *Trichogramma* or *Trichogrammatoidea* egg parasitoid species has no effect on naturally existing common natural enemies in the rice ecosystem as per the data in this study. It further confirms the suitability of egg parasitoids (*Trichogramma* or *Trichogrammatoidea*) as biocontrol agents to use in augmentation and release programmes in managing RLF control in Sri Lankan rice fields.

CONCLUSION

The findings are valuable to promote *T. bactrae* as a biocontrol agent to manage RLF.

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