

Egg Parasitoids of Cabbage Caterpillars in Sri Lanka: A Search for Biocontrol Agents

S.A.A. Singhamuni¹, K.S. Hemachandra^{2*} and P.G.A.S. Warnasooriya²

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ABSTRACT

Purpose : Management of cabbage caterpillar complex through augmentative release of egg parasitoids has been suggested considering the current issues in insecticide application, mainly the effect on human health, non-target effects and environmental pollution. Use of local egg parasitoid species is preferred considering the environmental risk associated with the use of exotic species; therefore, this study was conducted to examine the egg parasitoid guild of cabbage caterpillars in Sri Lanka.

Research Method : Field survey was conducted during January 2012 to May 2013 in major cabbage growing areas in the mid-country. Eggs of cabbage caterpillars were collected and reared them until the emergence of host larvae or adult parasitoids. Emerged parasitoids were preserved in 70% ethanol, and prepared the microscopic slides upon the dissection of male genitalia. Parasitoids were identified by using taxonomic keys and *Trichogramma* literature as per the protocol suggested by Natural History museum, London and the identities were confirmed by the taxonomist in Natural history museum in London.

Findings : *Trichoplusia ni*, *Spodoptera litura*, *Crociodolomia pavonana* and *Plutella xylostella* were found as the members of cabbage caterpillar complex. Two egg parasitoid species: *Trichogramma chilonis* Ishi and *Trichogramma achaeae* Nagaraja and Nagarkatti were found attacking eggs of cabbage caterpillars. *Trichogramma chilonis* has been reported previously in Sri Lanka but *Trichogramma achaeae* is the first country report. *T. chilonis* was widely distributed and caused for higher level of parasitism than *T. achaeae*. The highest level of parasitism (67%) by both species was found in *Trichoplusia ni* eggs which was significantly different from other species. Level of parasitism of caterpillar eggs was significantly varied between pesticide free fields (16.8%) and conventional fields (13.1%).

Research Limitation : The study of behavioral characters of these parasitoids is required to assess the suitability as biocontrol agents; as an initial step for augmentative biocontrol, the locally available egg parasitoids were reported in this study.

Originality/ Value : These findings would be contributing Sri Lankan agriculture sector to develop the augmentative biocontrol approach to manage the cabbage caterpillar complex and to produce pesticide residues free crop produce.

Keywords: *Crociodolomia pavonana*, Parasitism, *Plutella xylostella*, *Spodoptera litura*, *Trichogramma* spp., *Trichoplusia ni*

INTRODUCTION

Brassicas, including cabbage, radish, knolkohl (kohlrabi), and cauliflower are major vegetable crops in Sri Lanka. Of these, cabbage, which is most widely cultivated, is grown on over 4000 ha. Insect attack on cabbage is a major production constraint. Serious damage is caused by the cabbage caterpillar complex,

¹ Postgraduate Institute of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka

^{2*} Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka

ks_hemachandra@agri.pdn.ac.lk

 <http://orcid.org/0000-0002-1968-5105>

which includes the larvae of diamondback moth (*Plutella xylostella* Linnaeus (Lepidoptera: Plutellidae)), cabbage semi-looper (*Trichoplusia ni* Hübner (Lepidoptera: Noctuidae)), cabbage cluster caterpillar (*Crociodolomia pavonana* Fabricius (Lepidoptera: Crambidae)), and cabbage webworm (*Hellula undalis* Fabricius (Lepidoptera: Crambidae)) (Ketipearachchi *et al.*, 1992). Aphids (*Brevicoryne brassicae* (Linnaeus) (Hemiptera: Aphididae)), shield bugs (*Bagrada hilaris* (Burmeister) (Hemiptera: Pentatomidae)) and cutworms (*Agrotis* spp. (Lepidoptera: Noctuidae)) also cause damage.

Managing of the cabbage caterpillar complex is a challenge for brassica growers and currently foliar insecticide applications are commonly used. Department of Agriculture has recommended 13 insecticides for the control of cabbage caterpillar complex e.g; Chlorfluazuron 50g/l EC, Etofenprox 100g/l EC and Emamectin benzoate 5% SG (Department of Agriculture, 2015). Development of insecticide resistance, secondary pest outbreaks and pest resurgence are likely problems arising from excessive insecticide use (Van den Bosch *et al.*, 1982). Environment pollution and pesticide residues on crop harvest lead to human health problems; hence, minimizing of insecticide application on crucifer crops has been proposed.

Biological control is an important component of IPM and it is considered as safe, environmentally friendly, economical, and sustainable approach (DeBach, 1964). The three main approaches to biological control of arthropods are classical, augmentative release and conservation control (Bale *et al.*, 2008). Of the three, augmentation and release of indigenous parasitoids appears to be the most applicable approach for management of the cabbage caterpillar complex in Sri Lanka, as it poses less risk to non-target organisms and is logistically simpler. Some researchers have examined the parasitoid fauna in vegetable pests within the country (De Silva, 1961; Ketipearachchi, 2002; Nagalingam *et al.*, 2007). Locally available parasitoids of cabbage-feeding Lepidoptera have been documented (Jasudasan and Yogaratnam, 1984). However, a major limitation of using larval and pupal parasitoids in cabbage caterpillar control is that

plant damage occurs before parasitoid-induced mortality occurs. Thus, using egg parasitoids to kill members of the cabbage caterpillar complex at the egg stage is a desirable objective.

Egg parasitoids (*Trichogramma* spp.) have been successfully used in more than 56 countries and over 32 million ha are being treated with *Trichogramma* spp. to manage lepidopteran insects (Hassan, 1993). *Trichogramma* spp. have a worldwide distribution and about 145 species have been described in 80 genera (Pinto and Stouthamer, 1994). Augmentation and release of *Trichogramma* spp. has been successful in many other crops such as cotton, sugar beet, tomato, apple etc; therefore, cabbage insect control using egg parasitoids is suggested (Consoli *et al.*, 2010).

The objective of this study was to identify local egg parasitoids of cabbage caterpillars in Sri Lanka, as a first step towards their use in a biological control programme through augmentative release. Local parasitoid species are expected to be well adapted to the local climate and habitat (Hassan, 1989). Once candidate egg parasitoids are identified, the most suitable species for augmentative releases can be selected on the basis of the ease of mass rearing, their efficacy against target hosts, and their survival under local cultivation systems. In Sri Lanka, parasitoid augmentation has not been widely implemented but there is potential for farmers to operate their own programmes of mass rearing and release.

MATERIALS AND METHODS

This study was conducted from January 2012 to May 2013, and was based in the Department of Agricultural Biology, University of Peradeniya, Sri Lanka. The study has three major components: (a) Sampling of lepidopteran eggs in the cabbage ecosystem (b) Laboratory rearing of the collected eggs (c) Preparation of slides of adult parasitoids and identification of species.

Sampling of eggs in the cabbage ecosystem

Field surveys were conducted in selected cabbage

fields in vegetable growing areas at six locations (Thalathuoya, Galgediyawa, Alawathugoda, Hindagala, Ihalawela and Dodangolla) within the mid country (300 - 900 m mean sea level) of Sri Lanka. Selection of fields for sampling was based on the level of insect infestation, accessibility for regular sampling, cooperation of farmers and pattern of insecticide use. Cabbage fields were selected in two cultivation systems: conventionally managed fields receiving foliar insecticides, and pesticide-free fields that received no insecticide applications.

Selected fields were examined at weekly intervals, and collections were made of the eggs of brassica-feeding Lepidoptera. In addition, eggs of beneficial arthropods; coccinellids and spiders, were collected to assess the non-target effects of egg parasitoids. Egg collections were made by examining the cabbage plants, using a hand lens as required. When eggs were found they, and a surrounding disc of leaf were removed from the plant using a cork borer (20mm diameter). Each of the leaf discs with attached eggs was placed separately in a clear plastic vial (2.5cm diameter, 5.5cm height), labeled with a code which referenced the field collection data. Eggs from

each cabbage plants were placed in separate vials with further separation by caterpillar species. Identity of the caterpillar species was based on morphological characteristics of eggs or egg masses. The collected eggs were temporally stored in a cooler pack and transported to the laboratory.

Laboratory rearing of collected eggs

In the laboratory, eggs were identified using morphological characteristic examined at magnification x50 under a dissecting microscope (Meiji, Japan). Host eggs or egg masses when eggs could not be separated, were individually placed in clear plastic vials (2.5cm diameter, 5.5cm height). Vials were maintained at room temperature and examined everyday for the emergence of parasitoid adults or host larvae. Emerged parasitoid adults were separated and preserved in 70% ethanol for identification, attaching all collection data. In addition, the number and identity of adults emerged from each host was recorded in cases where superparasitism or multiparasitism was observed.

Table 01: Details of cabbage fields where host eggs were collected for assessment of egg parasitoid communities.

Pest control regime	Site name	Longitude and latitude	Sampling period	Site information
Conventional	Thalathuoya	7°14'30.95"N 80°40'32.15"E	January, 2012-May, 2013	Conventional vegetable cultivation, surrounded by perennial trees,
	Galgediyawa	7°10'48.31"N 80°35'8.20"E	January-June, 2012	Conventional vegetable cultivation, bordering to vegetable cultivations
	Alawathugoda	7°26'11.51"N 80°34'22.34"E	May-November, 2012	Conventional vegetable cultivation, Surrounding environment consisted with fallow field.
	Hindagala	7°14'2.11"N 80°36'4.72"E	April-August, 2012	Conventional vegetable land, surrounding with paddy
Pesticide-free	Ihalawela	7°10'7.18"N 80°34'39.37"E	February-May, 2012	Pesticide free vegetable cultivation surrounded by paddy land and manioc.
	Dodangolla	7°17'25.13"N 80°42'11.48"E	May-July, 2012	Pesticide free vegetable cultivation surrounded by teak hedge row and cultivated vegetable land.

Preparation and identification of adult parasitoids

Adult egg parasitoids were identified according to the protocol described by Noyes (2016). The male adult parasitoids were separated under the microscope on the basis of antennal characteristics, and carefully card mounted and preserved as dry specimens. Using the external morphology, different species were identified and series were prepared.

Card mounted specimens were mounted on microscope slides using the procedure of Platner *et al.*, 1999. The wings were separated and mounted in Canada balsam on a glass slide. The remainder of the insect was boiled in 10% potassium hydroxide solution for 5 minutes, and then raised through an ethanol dehydration series to absolute ethanol before transferring to clove oil. The dehydrated specimen was dissected in thin balsam on a glass slide under the dissecting microscope. The antennae, head capsule, wings, body and the genitalia were separately mounted in balsam under 8mm diameter coverslips. Prepared slides were kept at 35°C in an oven for 10 days until the slides were completely dry.

Slides were examined under a compound light microscope (x400) and the structure of the genitalia was compared with the illustrations in *Trichogramma* identification keys and the original descriptions. Species identities were confirmed on morphological characteristics by sending representative specimens to the Natural History Museum, London, U.K.; this was followed by verification using molecular techniques at University of California, U.S.A.

Data analysis

Percent parasitism was estimated as the number of parasitoid adults divided by the total number of host eggs multiplied by 100 (Van Driesche, 1983). The data were analyzed by using Chi square tests in SYSTAT 11, with an alpha level for significance of 0.05.

RESULTS AND DISCUSSION

Eggs of four cabbage feeding caterpillar species: *T. ni*, *S. litura*, *C. pavonana* and *P. xylostella* were found in the sampled cabbage fields (Table 02). *Trichoplusia ni* and *C. pavonana* were found in all fields. *T. ni* and *C. pavonana* were more abundant. *Plutella xylostella* appears to be uncommon though it has been reported as a common cabbage pest in Sri Lanka. The relative abundance of species of the cabbage caterpillar complex may vary with geographical location, time of the year and seasonal variation in host plant availability in an area. *Hellula undalis*, a member of the cabbage caterpillar complex, was not found during this study. The lepidopteran eggs collected in this study had been parasitized by two egg parasitoid species: *Trichogramma chilonis* Ishi (Hymenoptera: Trichogrammatidae) and *Trichogramma achaeae* Nagaraja and Nagarkatti (Hymenoptera: Trichogrammatidae). *Trichogramma chilonis* was observed parasitizing *Homona coffearia* (Nietner) (Lepidoptera: Tortricidae) eggs in Sri Lankan tea plantations in 1969 (Nagarkatti and Nagaraja, 1971) but, until our study, there had been no formally published reports of the species in the country since. *Trichogramma achaeae* has never been reported in Sri Lanka; hence, this is the first country report. Egg parasitoids have not been well studied in Sri Lanka. Only 10 species belonging to the Family Trichogrammatidae have been recorded (Noyes, 2016).

During this study, a total of 19,920 host eggs of cabbage caterpillar were collected. Of these eggs, 2,877 eggs had been parasitized, an overall level of parasitism of 14.44%. The level of parasitism of all host eggs by all parasitoid species varied significantly among the six sampling sites ($\chi^2 = 627.8$, $df = 5$, $P < 0.001$) and the total parasitism of host eggs by all species of parasitoids was in conventional fields was lower than in pesticide-free fields ($\chi^2 = 57.0$, $df = 1$, $P < 0.001$).

Table 02: Collected eggs of cabbage caterpillars and egg parasitoids that emerged from them.

Pest control regime	Site name (number of samples)	Host species	Number of collected host eggs	Number of parasitized eggs	Total parasitism (%)	Parasitism % by <i>T. chilonis</i>	Parasitism % by <i>T. achaeae</i>
		<i>Trichoplusia ni</i>	3029	1506	49.71	37.24	28.32
	Thalathuoya (16)	<i>Plutella xylostella</i>	34	11	32.35	32.35	0
		<i>Spodoptera litura</i>	3000	46	1.53	1.53	0
		<i>Crociodolomia pavonana</i>	2400	10	0.41	0.41	0
	Site totals and parasitism		8463	1573	18.59	12.1	8.0
		<i>Trichoplusia ni</i>	125	54	43.2	43.2	0
	Galgediyawa (7)	<i>Plutella xylostella</i>	60	5	8.33	8.33	0
		<i>Spodoptera litura</i>	1250	0	0	0	0
		<i>Crociodolomia pavonana</i>	1050	0	0	0	0
Conventional	Site totals and parasitism		2485	59	2.37	2.3	0
		<i>Trichoplusia ni</i>	346	121	34.97	34.97	0
	Alawathugoda (5)	<i>Plutella xylostella</i>	0	0	0	0	0
		<i>Spodoptera litura</i>	750	0	0	0	0
		<i>Crociodolomia pavonana</i>	750	0	0	0	0
	Site totals and parasitism		1846	121	6.55	6.5	0
		<i>Trichoplusia ni</i>	15	8	53.33	53.33	0
	Hindagala (4)	<i>Plutella xylostella</i>	0	0	0	0	0
		<i>Spodoptera litura</i>	0	0	0	0	0
		<i>Crociodolomia pavonana</i>	600	0	0	0	0
	Site totals and parasitism		615	8	1.30	1.3	0
Totals and parasitism for conventional sites			13409	1761	13.13	8.9	4.91
		<i>Trichoplusia ni</i>	1301	876	67.33	67.33	0
	Ihalawella (11)	<i>Plutella xylostella</i>	77	21	27.2	27.2	0
		<i>Spodoptera litura</i>	2250	5	0.22	0.22	0
		<i>Crociodolomia pavonana</i>	1650	0	0	0	0
Pesticide-free	Site totals and parasitism		5278	902	17.09	18.0	0
		<i>Trichoplusia ni</i>	326	214	65.64	59.27	31.28
	Dodangolla (9)	<i>Plutella xylostella</i>	7	0	0	0	0
		<i>Spodoptera litura</i>	194	0	0	0	0
		<i>Crociodolomia pavonana</i>	706	0	0	0	0
	Site totals and parasitism		1233	214	17.36	11.3	6.3
Totals and parasitism for pesticide-free sites			6511	1116	17.14	16.67	1.33

Parasitism of host eggs by *T. chilonis* occurred in all sites, and the level of parasitism of all host species was 11.5%. Parasitism of all host eggs by *T. chilonis* was significantly different among sampling sites ($\chi^2 = 527.41$, $df = 5$, $P < 0.001$). *Trichogramma chilonis* parasitized the eggs of all recorded caterpillar species at Thalathuoya. In all sites, *T. ni* was the species with the highest level of parasitism (Table 02). Parasitism of host eggs by *T. chilonis* was significantly influenced by pest control regime ($\chi^2 = 256.71$, $df = 1$, $P < 0.001$): in pesticide-free fields parasitism level was 16.67% and in conventional fields it was 8.9%.

Trichogramma achaeae was found only at Dodangolla and Thalathuoya (Table 02); and was recorded only from *T.ni* eggs. Level of total

parasitism of host eggs by *T. achaeae* in pesticide free fields was 1.33% and conventional fields 4.91% — a significant difference ($\chi^2 = 134.01$, $df = 1$, $P < 0.001$).

Trichogramma chilonis, was present in all sampling sites regardless of farming and pest management practices; this may be an indication of its adaptability and potential to be effective in diverse farming systems. Further, *T. chilonis* attacked all species of cabbage caterpillars that were present in each location indicating the host range.

Total parasitism of *T. ni* eggs was relatively high when both parasitoids: *T. chilonis* and *T. achaeae* attack eggs as happened at Thalathuoya and

Dodangolla. This signifies the possibility of existence of both species in the same field and sharing of the resources. This is a good character in potential biocontrol agents.

Trichoplusia ni eggs had the highest level of parasitism among all host species found in sampling sites, and was parasitized by both *Trichogramma* species. Over all sites, the level of parasitism of *T. ni* eggs by both parasitoids was 54%. Level of parasitism of *T. ni* in pesticide free field and conventional fields were 67.0 and 54.1% respectively which was significantly different. Other host species were parasitized only by *T. chilonis*. Parasitism of *P. xylostella* eggs, averaged over all sites, was 20.7%, but was observed in only three sites. Parasitism by *T. chilonis* of *S. litura* eggs was observed in only two sites, and of *C. pavonana* eggs were seen only at Thalathuoya.

A total of 840 eggs of non-target organisms were collected from the cabbage fields sampled in this study. Of these, 62 were spider eggs, and the remainders were eggs of Coccinellidae. No *Trichogramma* parasitoids emerged from these eggs (Table 03).

The highest total parasitism was recorded in cabbage fields in Thalathuoya. Although the study site receives pesticides, it is surrounded by dense trees with rich undergrowth. The higher total parasitism percentage may be due to dispersal of parasitoids to the vegetable fields from these surrounding natural habitats. This finding provides evidence on importance of natural habitat for conservation of natural enemies. Cabbage fields at Ihalawella and Dodangolla had comparatively

high parasitism levels, probably because these fields were managed without pesticides. Fields at Alawathugoda, Galgediyawa and Hindagala fields were managed with frequent applications of pesticides, and had lower levels of parasitism. High levels of parasitism in pesticide free fields have been recorded for *Trichogramma pretiosum* on *Heliothis virescences* (Bull and House, 1983; Suh *et al.*, 2000; Vieira *et al.*, 2001; Brunner *et al.*, 2001; Takada *et al.*, 2001).

Levels of parasitism of *C. pavonana* and *S. litura* were low. This is associated with the morphology of the egg mass. Unlike *T. ni* and *P. xylostella* which lay eggs singly or in small groups, *C. pavonana* and *S. litura* lay eggs in masses. *Crociodolomia pavonana* laid flat eggs which overlap and are arranged in a single layer. Parasitoids attempt to parasitize these egg masses but are unsuccessful due to the physical barrier. A few parasitized eggs did not produce adult parasitoids because early eclosing larvae destroy the eggs.

As no non-target parasitism was recorded, both *T. chilonis* and *T. achaeae* appear to be reasonably safe parasitoids to promote in the cabbage ecosystem to manage the cabbage caterpillar complex. The wider distribution and relatively high level of parasitism of *T. chilonis* make it a better prospect to use in augmentation biological control than *T. achaeae*, although augmentation releases of both species would appear to be beneficial, particularly where the main pest species in the cabbage caterpillar complex is *T. ni*.

Table 03: Numbers of non-target arthropod eggs collected and levels of parasitism.

Pest control regime	Site name and number of samples	Host taxon	Number of host eggs collected	Number of emerged parasitoids
Conventional	Thalathuoya (16)	Coccinellid species	296	0
		Spider eggs	34	0
	Galgediyawa (7)	Coccinellid species	34	0
		Alawathugoda (5)	Spider eggs	6
	Hindagala (4)	Coccinellid species	21	0
Pesticide-free	Ihalawella (11)	Coccinellid species	321	0
		Spider eggs	22	0
	Dodangolla (9)	Coccinellid species	106	0

CONCLUSIONS

Four major cabbage caterpillar species: *Trichoplusia ni*, *Spodoptera litura*, *Crociodolomia pavonana* and *Plutella xylostella* were present in the sampled cabbage fields. *Trichoplusia ni* and *C. pavonana* were more abundant in cabbage crops in mid-country area. Two species of *Trichogramma*: *T. chilonis* and *T. achaeae* were present in cabbage cultivations parasitizing caterpillar eggs. *Trichoplusia ni* eggs were highly susceptible for the parasitism and *C. pavonana* and *S. litura* eggs were less liable to parasitism. *Trichogramma chilonis* was geographically widely distributed and parasitized comparatively higher number of host eggs than *Trichogramma achaeae*. *Trichogramma chilonis* most of the time tolerates conventional farming practices

than *T. achaeae*. Non target effect of *T. chilonis* or *T. achaeae* was not detected; hence, there is a high potential to control cabbage caterpillars, particularly *T. ni* by using locally available parasitoid species: *T. chilonis* and *T. achaeae*.

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Declaration of interest statement

The authors declare no conflicts of interest.

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