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Biology and life history of *Cotesia ruficrus* (Hymenoptera: Braconidae) a potential parasitoid of *Hyposidra talaca* (Lepidoptera: Geometridae) larvae, a major tea pest

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ABSTRACT

The black inch looper, *Hyposidra talaca* is considered as a major pest in tea in northern part of West Bengal and North East India. Among the natural enemy reported, *Cotesia ruficrus* is considered as one of the most gregarious endo-parasitoid wasps. In order to assess the potential of this natural enemy, a study on the biological parameters of *C. ruficrus* was evaluated on the different developmental stages (second, third and fourth instars) of the host larvae, *H. talaca*. The results indicated that, the mean duration of larval development was 12.0 ± 0.32, 11.0 ± 0.45 and 9.2±0.37 days in second, third and fourth instar host larvae respectively. The pupal period of *C. ruficrus* was found to be significantly different among the different larval stages of *H. talaca*. The successful parasitism of *C. ruficrus* and the number of cocoon formation of the parasitic wasp was reliant on the stage, body size and the physiological conditions of host larvae that it parasitizes. A maximum of 65.2±1.85 cocoons were formed when the fourth instar host larvae parasitized, followed by 27.2±3.04 in the third instar and 4.6±0.68 in the second instar host larvae. The number of females and males hatched out from each clutch was compared to the different host stages. .

Key words: Black inch looper, *Hyposidra talaca*, Parasitic wasp, *Cotesia ruficrus*, Tea, Parasitism.

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INTRODUCTION

The black inch looper, *Hyposidra talaca* (Lepidoptera: Geometridae), considered as a major tea pest, which causes considerable damage to tea plantations leading to loss in yield and the quality of manufactured tea as well. In general, chemical insecticides are being used for the control of tea pests including this black inch looper (Hazarika *et al*., 2009) by the tea planters. Hence the use of chemical pesticides has many negative impacts such as development of resistance in insects, abolition of natural enemies, imbalance in natural ecosystems and increasing environmental contamination besides causing ill-health to human beings. Therefore, non-chemical control measures are extremely important for the management of this destructive pest (Deka *et al*., 2017; Nguyen *et al*., 2018). Biocontrol agents play a vital role for the management of pests, especially lepidopteran pests with a well-balanced ecological systems, by helping in the reduced use of pesticides in major agricultural crops, including tea.

The tea ecosystem, which is considered as a semi-forest ecosystem, harbor more than 1034 arthropod species associated within (Hazarika *et al*., 2009). Among the diversity of arthropod natural enemies in sub-Himalayan tea growing region of northern part of West Bengal, parasitoid groups belonging to the families such as Braconidae and Ichneumonidae are dominant ones. Among the parasitoids, *Cotesia ruficrus* (Haliday) is found to be one of the effective parasitoid wasps which attacks on tea looper complex (Das *et al*., 2010). Several innovative research works have been done on the host- parasitic interaction between *C. ruficrus* and *Cnaphalocrocis medinalis* (Chen *et al*., 2016) *Agrotisipsilon, Helicoverpa armigera, Mythimna separate, Spodopter alitura, S. exigua* and *Exelastis atomosa* (Hill, 1986; Patil *et al*., 2016).The parasitoid, *Cotesia* spp was reported to be an efficient parasitoid on its selected host *H. talaca*, consequently leading to the death of the host (Das *et al*., 2010). Just after emerging from the host, the larvae of the parasitic wasp construct pulpal cocoons and after few days, the adults emerge out (Potting *et al*., 1997). In the present study, an attempt has been made to investigate the biological parameters of *C. ruficrus* on different larval stages of *H. talaca* besides assessing it's the impact on the larvae of *H. talaca*, *under* laboratory conditions.

MATERIALS AND METHODS

Maintenance of *H. talaca* and *C. ruficrus*

Healthy male and female moths of *H. talaca* were collected from TRA (Tea Research Association) experimental plot (26.8809°N 88.9079°E), kept in mating and egg laying chamber (glass chimney) with 10% honey solution as food source. Eggs were kept in a separate chamber and after hatching, larvae were reared on selected susceptible tea clone under the laboratory conditions (23± 2^{0}C, 68-77% RH and 13:11 L:D photoperiod), methodology adopted from Sarkar *et al.* (2019). Cocoons of *C. ruficrus* were collected from a nearby tea garden (Nagrakata Tea Estate, 26.9163°N 88.9079°E) and were kept in a glass chimney and after adult emergence 10% honey solution was provided as food to the adult parasitoid wasps. Different life stages of larvae of *H. talaca* were exposed to the adult parasitoids for parasitism. Then, the parasitized host larvae were separately put into a glass chimney containing tea leaves for their feeding. The parasitoid larvae, after emergence from the parasitized looper construct a cocoon clutch. The cocoons were collected and put separately, by following the method of Chen *et al*. (2016).

Biological parameters of *C. ruficrus*

In order to record the biological parameters after parasitization, the developmental period of parasitoid larvae inside the host, pupal duration, number of cocoons formed, the adult longevity of parasitic wasp and the number of male and female wasps emerging from each parasitized larvae were observed on a daily basis. Total 10 host larvae were used in each experiment and replicated five times, followed by the methodology of Robert *et al.,* (1992) with minor modifications.

Parasitic capacity of *C. ruficrus*

First, second and third instar larvae of *H. talaca* and adult parasitoids of *C. ruficrus* were released into a glass chimney in the parasitoid/host ratio of 2:1 and parasitoid wasps female/male ratio was 2.17:1. After 4-6h of release, the host larvae were placed separately in a glass chimney along with food (tea leaves), and the opening of the jar was covered with muslin cloth. This experiment was continued until the death of released female parasitoids. Host larvae were observed either until the host pupate or until the emergence of parasitoid larvae. Among the parasitized host larvae, number of death and formation of pupa were observed (Kaiser *et al*., 2017).

Statistical analysis

Data recorded from the study of parasitism were statistically analyzed by chi-square statistic method at 5% level of significance. Biological parameters of *C. ruficrus* were analyzed by One-way ANOVA test and the means were separated by Tukey's multiple comparison test.

RESULTS AND DISCUSSION

Biological parameters of *C. ruficrus*

The results obtained from the studies on the biological parameters indicated, *C. ruficrus* successfully parasitized and completed its life cycle in the second, third and fourth instar larvae of *H. talaca* (Table 1). The duration of developmental period was found to be higher in second instar followed by third and fourth instars. Similar trends have been observed, as in the case of earlier workers Khan *et al*. (2017) and Omwega and Overholt (1997), on

Table 1. Biological parameters of *C. ruficrus* reared on *H. talaca* larvae under laboratory conditions.

Biological parameters	Life stages of *H. talaca* (Host)					
	II instar	III instar	IV instar	F-statistic value	P-value	CD (0.05)
Larval development period (Days) *	12 ± 0.32^{a}	11 ± 0.45^{a}	9.2 ± 0.37^{b}	13.726	0.00079	1.384
Pupal development period (Days) *	5.8±0.20^{a}	4.6±0.51^{ab}	4.0±0.45^{b}	5.039	0.0257	1.358
Adult female longevity (Range)	4 to 6 days					
Adult male longevity (Range)	3 to 4 days					
Sex ratio (F:M)	1:0.5					
Total developmental period (Range)	19 to 24 days					

*Values are represented as mean ±SE of five replications followed by the same letter in a row do not differ significantly.

the biology of *C. flavipes* and *C. sesamiae* reared on different larval instars of *Chilo partellus* and Gramineous stem borers under laboratory conditions. In second instar host larvae, the mean number of cocoon formation was comparatively very less compared to the other stages, may be because of the body size and stage of the host larvae. A mean number of 65.2±1.85 cocoons per fourth instar host larva, 27.2±3.04 cocoons per third instar host larva and 4.6 ± 0.68 cocoons per second instar host larva were formed. Which were significantly different from each other (*F*=214.119 and *P*=0.00) (Fig. 1). This result is in well agreement with the study by Khan *et al*., (2017) on the biological parameters of *C. flavipes* on different larval instars of *Chilo partellus*. Omwega and Overholt, (1997) also observed that, the production of progeny of *C. flavipes* were more in the large sized host larva of gramineous stem borers and less in medium and small size larvae. Pupal development of *C. ruficrus* was also varied with different host instars (Table 1). Pupae formed from the second instar host larvae have taken more time to complete the pupal duration compared to third and fourth instar host larvae. Kaiser *et al*., (2017) also reported similar variations in the pupal duration of *C. typhae* of France strain. Chen *et al*., (2016) also reported the maximum number of cocoons / clutches of *C. ruficrus* in fourth instar host larvae of *C. medinalis* followed by the third and second instars.

The number of adult females were recorded more when the number of pupae is more. Out of the 65.2±1.85 cocoons, 47.6 ± 2.50 females and 17.4 ± 2.18 males were found in the fourth instar host larvae (Fig. 1), which was equivalent to the sex ratio of 1:0.5 (*F*= 122.734 and *P*=0.00).

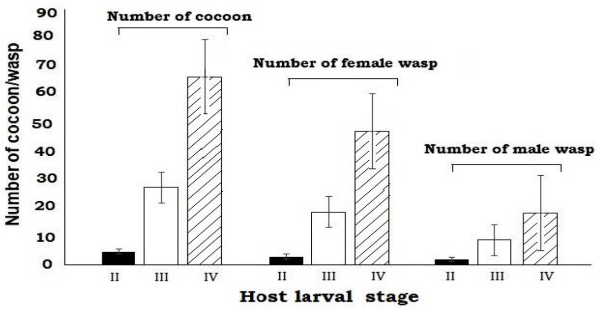

Fig. 1. Number of cocoon formation of *C. ruficrus*, adult female and male wasp emergence from a single host larva from different stages. Values are significant at P <0.05 (One-way ANOVA).

Whereas the number of females and males in the third instar host larvae was found to be significant (*F*= 34.239 and *P*=0.00001). In the case of second instar host larva, the number of pupal cocoons formed was comparatively less. Jiang *et al*. (2004) explained the same strategy when *C. flavipes* parasitizes on different larval stages of *Chilo partellus*. Emergence of a maximum number of female wasps from a larger cocoon clutch with high sex ratio of (female:male) compared to that from a smaller clutch in different species of *Cotesia* on different host species (Kaiser *et al*., 2017; Omwega and Overholt, 1997;Potting *et al*., 1997; Robert *et al*., 1992). The adults of *C. ruficrus*, reared by providing 10% honey as food in order to record the longevity. The mean longevity of the adult male was ranged between 3 to 4 days, whereas female longevity was recorded as a rage of 4 to 6 days. Potting *et al.* (1997) showed that the longevity of *C. flavipes* was about 5-6 days when honey solution used as food. Kaiser *et al*., (2017) assessed the life span of *C. typhae* using honey and saccharose solutions as food and observed more life span of adult wasp while fed on honey. Whereas, Muirhead *et al*. (2008) reported that, the average longevity of *C. nonagriae* was about 12days.The total developmental duration were ranged from 19.2 to 23.8 days (Table 1). From the parasitism to the parasitic larval emergence (Fig. 2), this period was found to be variable among three different host larvae, which is almost similar with the study of Khan *et al*. (2017) and Omwega and Overholt, (1997) on the biology of *C. flavipes* and *C. sesamiae* reared on different larval instars of *Chilo partellus*.

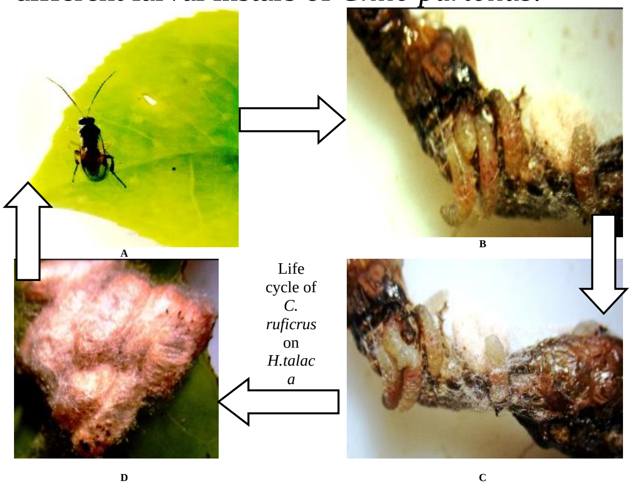

Fig. 2. Life cycle of *C. ruficrus* on host larva of *H. talaca*. **(A)** Adult of *C. ruficrus*. **(B)**Emergence of larvae of *C. ruficrus* from host larva. **(C)**Initiation of cocoon formation. **(D)** Clutch of cocoon formed by the wasp's larvae

Parasitism by *C. ruficrus*

The stage specificity for successful parasitism by *C. ruficrus* has been evaluated by providing different host larval instars of *H. talaca*. The highest percentage parasitism of almost 74% with maximum number of pupal cocoons were recorded from the fourth instar host larvae when compared with the other two larval stages (Table 2). In the current investigation, the less number of females emerging from the early stages of the host larva indicates the stage specific preference of *C. ruficrus* towards the later stages, for the successful parasitism of *C. ruficrus* on *H. talaca*.

Table 2. Successful parasitism of *C. ruficrus* on the different stages host larvae of *H. talaca*

Parameters	Life stages of *H. talaca* (Host)		
	II instar	III instar	IV instar
Parasitization of host (%)	40	58	74
Host pupation (%)	42	30	16
Mortality of host after parasitization (%)	18	12	10

The chi-square statistic is 24.24. The result is significant at $p < .05$.

The female wasps show more preference towards the fourth instar host larvae of *H. talaca*, more for successful parasitization may be attributed to the large body size which could be more suitable for the developing larvae inside the host. Similar parasitic activities were also reported earlier by Chen *et al*. (2016) and Hill (1986), on the host selection of *C. ruficrus*. The early stages of *H. talaca* could not support successful parasitism by *C. ruficrus*, which was evident from the highest mortality of the host larvae (18%) and less number of progeny when compared to the other two tested larval stages. Example of successful parasitism by *C. ruficrus* and low host mortality were shown by Patil *et al*. (2016). Rahman and Bhola (2011) reported about 30-80% successful parasitism of *Apanteles taprobene* on the major tea looper *Buzura suppressaria*.

From the present study, it is evident that, *C. ruficrus* has shown the ability to overcome the immune system of the larvae of *H.talaca* and developed successfully. The results of the experiment also revealed that, more than 60% successful parasitism of *C. ruficrus* on fourth instar host larvae which indicated that *C. ruficrus* has the ability to regulate the population of this major tea pest in tea ecosystem. The host -parasitic interaction between *C. ruficrus* and *H. talaca* might be helpful in developing an IPM component for an ecofriendly management. Conservation of this gregarious parasitoid wasp will be beneficial in enhancing the natural enemy population in tea ecosystem.

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