SEASONAL OCCURRENCE OF EGG PARASITOIDS AND PARASITISM OF *OOENCYRTUS* SP. ON *RIPTORTUS PEDESTRIS* (HEMIPTERA: ALYDIDAE) EGGS

A THESIS

BY

SHARMIN AKTER

Student No. 1805240

Session: 2018-2019

Thesis Semester: July-December, 2019

MASTER OF SCIENCE (MS)

IN

ENTOMOLOGY

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HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR

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DEDICATED

TO MY

BELOVED PARENTS

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The author

ABSTRACT

Riptortus pedestris (Hemiptera: Alydidae) is one of the most important pests of various crop plants in Korea and Japan and a newly introduced pest of various leguminous crops in Bangladesh. In this study, the seasonal abundance of *R. pedestris* and its egg parasitoids were observed in three different locations in Dinajpur and biological attributes of *Ooencyrtus* sp. (Hymenoptera: Encyrtidae) were studied in laboratory condition. Eggs, nymphs and adults of *R. pedestris* were observed in three different study locations. *R. pedestris* were captured by aggregation pheromone traps during the study period. Gregarious parasitoids, *Ooencyrtus* sp. and solitary *Gryon nigricorne* (Hymenoptera: Scelionidae) emerged from natural eggs. *Ooencyrtus* sp. and *G. nigricorne*were observed during the whole study time. *Ooencyrtus* sp. displayed drumming, drilling, feeding and oviposition behaviors and increased time spent in newly emerged fresh host eggs and decreased when the host eggs age was increased. *Ooencyrtus* sp. can successfully parasitize fresh host eggs and up to 48 hours and produced 22 parasitoids /female within 24 hours. The fresh host eggs of 96-hour, parasitism decreased by approximately 29% compared to the control. Emergence rate and sex ratio were not affected by the age of fresh host eggs. No eggs were hatched after 30 days of refrigeration. In addition, none of the attributes of *Ooencyrtus* sp. was negatively affected by host eggs refrigerated up to 30 days. Refrigeration of host eggs was not affected in emergence rate and sex ratio of this gregarious egg parasitoid. Therefore, fresh and refrigerated eggs of *R. pedestris* can be successfully parasitized by *Ooencyrtus* sp. and refrigeration of host eggs can be a good alternative for the mass rearing of *Ooencyrtus* sp.

Key words: *Ooencyrtus* sp., *Riptortus pedestris*, Egg parasitoid, Bean bug, soybean, mass rearing, Biological attributes.

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CHAPTER I

INTRODUCTION

Riptortus pedestris (Hemiptera: Alydidae) is a polyphagous and economically important pest of various crop plants such as soybean, sesame, bean, rice, apple, sweet persimmon, jujube in Korea and Japan (Honda,1986; Son *et al.*, 2000; Lee *et al.*, 2004; Kang *et al.*, 2003) and a newly introduced pest of many leguminous crops in Bangladesh (Alim 2018). It is also a serious pest of many grains like barley, foxtail millet, sorghum in the reproductive stages (Chung *et al.*, 1995; Mainali *et al.*, 2014), as well as other field crops in Asian countries (Kang *et al.*, 2003; Wada *et al.*, 2006). Both adults and nymphs of *R. pedestris* feed preferentially on seeds by piercing and sucking (Choi *et al.*, 2005). It is one of the major Hemipteran pests of soybean (Osakabe and Honda, 2002; Choi *et al.*, 2005) and fruit trees (Chung *et al.*, 1995). The bug feeds mainly on leguminous plants and pods cause serious damages. Both adults and nymphs bore the pods and feed on the juice so that the pods fail to mature, turn brown, shrivel and die. When such pods are opened, then shriveled, undersized, and malformed seeds have appeared. Such seeds easily succumb to secondary infections like fungal infection, and poses problems during milling and post-harvesting processes. The damage caused by *R. pedestris* is especially serious in soybean because they feed on pods (Son *et al.*, 2000; Choi *et al.*, 2005) and transmit yeast-spot disease (Kimura *et al.*, 2008).

Legume growers spray insecticides to control *R. pedestris* but it is not economical because bean is a low-income crop. However, insecticide spray become more convenient and shows quick action. *Riptortus pedestris* is difficult to control due to its high mobility (Choi *et al.*, 2005; Wada *et al.*, 2006) and broad host range. In addition, chemical insecticides not only increase production cost, but result in the reduction of beneficial insects, pest resurgence, and other environmental problems (Shepard *et al.*, 1977; Orr, 1998; Schwab *et al.*, 1995). Cultural control methods such as delayed seeding and use of resistant varieties have been suggested in the stink bugs management program (Bowers, 1990; Wada *et al.*, 2006). Therefore, sounder pest management strategies such as biological control should be employed. Egg parasitoids are good candidates for the biological control of various stink bugs and have been used successfully in both augmentation and introduction (Orr, 1998; Weber *et al.*, 1996).

Riptortus pedestris eggs are attacked by *Ooencyrtus nezarae* Ishii (Hymenoptera: Encyrtidae), *Gryon japonicum* Ashmead (Hymenoptera: Scelionidae), and *Gryon nigricorne* Dodd (Hymenoptera: Scelionidae) in soybean fields (Noda, 1989; Hirose *et al.*, 1996; Mizutani *et al.*, 1996; Son *et al.*, 2009). *Ooencyrtus* sp. (Hymenoptera: Encyrtidae) and *Gryon nigricorne* is the major egg parasitoids of *R. pedestris* eggs in country bean fields in Bangladesh (Alim, 2019 Unpublish). *Ooencyrtus* sp. is a gregarious parasitoid and is widely distributed in Korea, Japan and Bangladesh (Mizutani *et al.*, 1996, Paik *et al.*, 2007; Alim, 2019 Unpublish). It can parasitize the eggs of 13 Hemipteran species including pentatomids pests on soybean such as *Piezodorus hybneri* Gmelin, *Dolycoris baccarum* Linnaeus, and *Nezara antennata* Scott (Hirose *et al.,* 1996; Mizutani *et al.,* 1996; Zhang *et al.,* 2005). Among them, *R. pedestris* eggs are the major host resources for *Ooencyrtus* sp. in country bean and mungbean fields (Alim, 2019 Unpublish). The parasitoid, *Ooencyrtus nezarae* has high dispersal ability (Hirose *et al.*, 1996) and its field parasitism on *R. pedestris* egg reached 58.5% which is six times higher than parasitism by *G. nigricorne* (Noda, 1989).

There is no research work has been done about the egg parasitoids of newly introduce insect *R. pedestris* in Bangladesh. Biological attributes of *O. nezarae* were studied in different countries. But biological parameters of *Ooencyrtus* sp. were still unknown in worldwide as well as Bangladesh. For successful biological control program, it is needs to know the different biological parameters of *Ooencyrtus* sp. as newly identified genus in Bangladesh. In this study, biological parameters of *Ooencyrtus* sp. on *R. pedestris* eggs were evaluated in laboratory condition.

Therefore, different biological parameters such as emergence rate, development time, sex ratio, adult longevity and size of the parasitoid were compared for different periods of time. Furthermore, survival percent of refrigerated *R. pedestris* eggs were also studied since these parameters are important for augmenting parasitism in the field. In addition, seasonal occurrences of egg parasitoids are observed in three locations to enhance the parasitism of the bean bug and thus reduce the pest population.

CHAPTER II

REVIEW OF LITERATURE

Riptortus pedestris is newly introduced pest in Bangladesh which may cause serious damage for different leguminous crops. Studies on the management of *R. pedestris* by using biological control agents such as Egg parasitoid are new in Bangladesh. There is no literature on this insect in Bangladesh but there are a few literatures found in Asian countries like Korea, Japan, China and India. However, available information on the *R. pedestris* has been taken as review of literature.

2.1 Soybean

Soybean (*Glycine max* L.), a native oilseed crop of East Asia, is a complete source of protein, and it also constitute significant amounts of nutritious and functional components such as phytic acid, alpha–Linolenic acid, and the isoflavones such as genistein and daidzein (Kwak *et al.,* 2007; Anderson and Wolf, 1995; Oh *et al.,* 2002). In Bangladesh about 85% of the total protein consumed came from plant sources. Soybean area and production levels for marketing year (MY 2018/19 July to June) in Bangladesh are projected to increase to 80,000 hectare and 152,000 tons respectively, due to a switch from Boro rice production. According to a USDA GAIN report, soybean planting area and production estimates for MY 2017/18 are lowered to 60,000ha and 114,000 tones due to unsuitable conditions for planting at the optimal time, and the shift of land to Boro rice cultivation due to an expected higher harvest price.

Meanwhile, MY 2018/19 soybean and soymeal imports are estimated to increase to 1.1 million tones and 550,000 tons respectively, to keep pace with demand in the feed industry, as well as growth in the livestock and fisheries sectors, the report said. Interest in soybean has increased in recent years in part because of their reported beneficial effects on human health (Adlercrereutz *et al.,* 1993; Lee *et al.,* 2008).

2.2 *Riptortus pedestris*

Riptortus pedestris Fabricius (Hemiptera: Alydidae), is one of the major hemipteran pests of soybean (Son *et al.,* 2000; Osakabe and Honda, 2002; Choi *et al.*, 2005) and fruit trees (Chung *et al.,* 1995) in Korea, Japan and newly introduced pest in Bangladesh (Alim 2018). The bug feeds mainly on leguminous plants and causes serious damage. The damage caused by *R. pedestris* is especially serious in soybean because they feed on pods by piercing and sucking (Son *et al.,* 2000; Choi *et al.,* 2005) and transmit yeast –spot disease (Kimura *et al.,* 2008). *Riptortus pedestris* pass three generation per year. First generation occurred from early July to early august, second generation from mid august to late September and third generation occurred in early October to mid November, entered reproduction diapauses without carrying eggs in the ovaries (Huh *et al.,* 2005).

Bae *et al.,* (2005) reported temperature dependent-development and oviposition of *Riptortus* sp. and found 28^{0}C as the optimum temperature for the development and oviposition. Hatchability was highest at 28^{0}C and decreased with increasing temperature. Nymph instar duration was 38 days at 20^{0}C, 30^{0}C days at 24^0 C, and 23 days at 28^{0}C. Adult emergence was 72% at 28^{0}C, and longevity was 63 and 60 days for female and male, respectively. Pre-oviposition duration ranged from 11 days at 20^0 C to 5 days at 32^{0}C. Total fecundity ranged from 21 eggs at 20^{0}C to maximum 67 eggs at 28^{0}C.

Taxonomic position of *R. pedestris*

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Hemiptera

Superfamily: Coreoidea

Family: Alydidae

Genus: *Riptortus*

Species: *R. pedestris* Fabricius

Nature of damage

Both adults and nymphs of *R. pedestris* mainly feed soybean pods and also consume leaves & stem of soybean plants (Ha *et al.,* 1998; Lee *et al.,* 2004). Major economic damage occurs when soybean plants are in R3 and R4 stages (i.e. beginning to full pod stage) (Kono, 1989; Jung *et al.,* 2005).

James *et al.*, (1982) reported that initial infestation with three leaf footed bug adults per plant during early bloom stages caused an average of 22% pod damage, average rate of seed abortion of 63% and an average rate of seed yield of 54%, whereas late bloom infestations sustained 22% loss in total seed yield in cow pea. In the forest zone and Guinea savana of Nigeria, yield losses attributed to Stink bug ranged from 25 to 60%. Jackai *et al.*, (1988) assessed damage caused by stink bugs and reported that seed damage caused by bugs ranged from an average of 32 to 59%. Son *et al.* (2000) reported that the damage caused by stink bugs in determinate types of soybean ranged from 5.0 to 12.5% and indeterminate types, the damage was 36.1 – 50.1%.

Distribution

Riptortus pedestris is widely distributed in Asian countries including Japan, China, Indonesia, and India (Kikuhara, 2005). *Riptortus pedestris* is a multivoltine insect having 2–3 generations per year in Korea and Japan (Kono, 1989; Shin *et al.*, 2012). Based on ovarian development of *R. pedestris* adults, Huh and Park (2005) reported that three generations per year would be possible in the southeastern region of South Korea. Lim (2013) observed that Hemipteran bugs, which were previously considered as secondary pests, have currently become important pests of numerous crops. Among them, *R. pedestris* is a major species that occurs in Korea, Japan, China, and South Asian countries. Kim *et al.* (2014) conducted an experiment to evaluated that *R. pedestris* start to emerge earlier in spring when reproduction is not possible due to scarcity never became of nutritious food sources, thus host exploitation pattern is not well known. In order to understand the patterns of seasonal occurrence and how it relates to suitability of host plants in the spring, the seeds of barley (*Hordeum vulgare* L.), sesame (*Sesamum indicum* L.), and black locust (*Robinia pseudoacacia* L.) was evaluated as pre-season food sources for *R. pedestris*. When nymphs fed sesame seeds only, *R. pedestris* never became third instars.

Seasonal Abundance of *R. pedestris*

Singh and Singh (1978) reported the highest incidence of 57 bugs per plant on early red gram variety BS1 in the 39th standard week of October and the population of pest attained its peak (9 bugs/10 plants) by middle of November. Lee *et al.* (2004) conducted experiment on seasonal occurrence of *R. clavatus* in soybean fields. They reported the peak occurrence of *R. clavatus* at late of August for late May seeding and middle of September for middle June seeding. Bharathimeena *et al.* (2008) conducted an experiment in India, during 2005–2006 to evaluate the seasonal incidence of pod sucking bugs infesting vegetable cowpea and their natural enemies. The nymph population of the alydid pod bugs, *R. pedestris* F. and *R. linearis* (F.) was significantly higher during the first fortnight of May 2006 and the succeeding three fortnights. Adult population of *R. pedestris* and *R. linearis* peaked significantly during the first and second fortnights of June 2006 respectively, when compared to most other periods of the year.

Mizutani *et al.* (2011) elucidated that the annual lifecycle of the *R. pedestris* Fabricius, especially its characteristics on potential host plants before it attacks soybeans, also investigated the seasonal outbreak of the bean bug in Chinese milk vetch, red clover, and soybean fields from 2004 to 2008. In Chinese milk vetch fields, adults of *R. pedestris* appeared from late April, and their progenies appeared from mid-May to mid-June. Adults and nymphs were found in red clover fields until late August to mid-October. In soybean fields, there were no differences in the immigration and population density of adults and nymphs by year. Following adults, nymphs appeared from late July to early August in soybean fields. Nymphs and adults of the next generation appeared on each host plant, indicating that *R. pedestris* can complete its annual lifecycle using Chinese milk vetch, red clover and soybean as successive host plants. Shin *et al.* (2012) investigated that seasonal fluctuations of *R. pedestris* were using aggression pheromone traps from April to November in 2010 and 2011. The population density of *R. pedestris* was high in mid-June, mid-August, and late October in 2010 and in early May, mid-June, mid-September, and early October in 2011 which were distinguished in farmlands and forests, were highest from June to August in the farmland and in September in the forest. Similar numbers of *R. pedestris* were capture in the farmlands and the forest in June-August, September-November, respectively. These results suggest that the insects did not migrate in August because food was plentiful in the forest at 200 m, but they moved to the forest during October due to the scarcity of food and for overwintering. The *R. pedestris* seasonal fluctuations in 2011 were affected heavily by the environment, particularly rain precipitation.

Host Range

Riptortus pedestris is highly mobile and has a wide host range, making field-by-field control difficult and inefficient (Park *et al.,* 2016). *R. pedestris* overwinters as an adult near soybean fields or under leaf litter in mountainous areas (Moriya, 2005). After overwintering, *R. pedestris* undergoes 1–2 generations on wild hosts (e.g. Chinese milk vetch) and then colonizes soybean fields in April (Mizutani *et al.,* 2011). *R. pedestris* causes the greatest damage to soybean, as it is the most suitable host for the bug (Lim, 2013). *R. pedestris* can consume leaves, stem, and pods of soybean (Ha *et al.,* 1998; Lee *et al.,* 2004).

The ornamental plant, *Caesalpinnia pulcherrima* and the weed, *Cassia occidentalis* were new reports of host plants of *R. pedestris* (Bharathimeena and Sudharma, 2009). The bean bug *R. pedestris,* known as a serious pest of leguminous crops, develops sac-like tissues called crypts at the posterior region of the mid-gut, the lumen of which is densely colonized by a *Burkholderia* symbiont belonging to the *Betaproteobacteria* (Kikuchi *et al.,* 2005).

Oviposition site of *R. pedestris*

Oviposition preference of *R. pedestris* was observed as plant within a field at different developmental stages, and to different leguminous germplasms. The insect laid its eggs mainly on the back surface of leaf in the upper half of plant in both observations from oviposition cage and soybean field. The egg numbers in fields were observed at a statistically-same rate in marginal and inside area, and at a median value of one egg per leaf in oviposited leaves. Full seed stage of soybean hardly affected oviposition preference of the insect (Jung, 2008). *R. pedestris* females oviposit not only on host plants but also on non hosts, which may impose high costs on nymphs in terms of locomotion energy and time searching for host plants (Nakajima *et al.*, 2010).

2.3. Pest management tactics for *R. pedestris*:

Botanical control of *R. pedestris*

Kwon *et al.* (2011) conducted an experiment while minimizing insecticide use for the environmental friendly agriculture, the control of the *R. pedestris* can be utilized environmental friendly agricultural materials (EFAMs) in the green manure crop fields. The control effects of some EFAMs and most of chemical pesticides to the *R. pedestris* were significantly high. As a result of direct spray of EFAM that contained neem extract, the control effects of six EFAMs were higher than 70% at 120 hours after treatments. Among them, three EFAMs were showed over 90% of control effects. On the other hand, most of chemical pesticides were showed 100% of control effect against the *R. pedestris* at 48 hours after treatment. As mortality effects of EFAMs were slowly observed until 120h after treatment, we must use selected EFAMs at the beginnings of occurrence and entrance in the field of green manure for effective control of *R. pedestris*.

Biological control:

Use of biological control agents against insect pests began over 1000 years ago in citrus grove to control caterpillar pests by the use of predatory ants (Huffaker *et al.,* 1971). The first successful commercial example of classical biological control dates back to 1888 when the *Vedalia* beetle was used to control the country cushion scale, *Icerya purchase* Maskell (Homoptera: Margorodidae) in California (Van Dresche and Bellows, 1996). In spite of efforts in managing pests through alternative methods like cultural, mechanical, and biological, chemical pesticides has been predominant due to its convenience and fast action.

In 1962 Rachel Carson published her book "Silent spring" which raised concern about the devastating effects of the continued use of chemicals on the environment. She reported concerns on the rates of chemical breakdown, toxicity of chemicals to non-target organisms, accumulation of chemicals in the food chain, as well as problems of insects becoming resistant to chemicals such as DDT. This changed the public view that chemicals provided the panacea for pest control.

Cultural control:

Cultural control methods such as delayed seeding and use of resistant varieties have been suggested in stink bug management program (Bowers, 1990, Masahiro *et al.,* 1990; Wada *et al.,* 2006). Relationship between seed damage by soybean bugs and timing of flowering were reported in Japan. Igita and Iwata (1979) found that later flowering cultivars and lines tended to have less seed damage by soybean bugs. The percentage of damaged seed in fields decreased as the flowering of soybean was progressively delayed (Yamanaka *et al.,* 1990). Thus, seed damage by soybean bugs seems to be avoidable in late-maturing soybeans in southern Japan.

Bowers (1990) registered the "Crockett" soybean cultivar resistant to the southern green stink bug (*Nezara viridula*). The cultivar IAC-100 and its progeny were resistant to several insect species including stink bugs complex preying on soybean crops (Rossetto *et al.,* 1995; Pinheiro *et al.,* 2005).

Chemical control

Historically, Sulfur is the first recorded insecticide used by the Sumerians to control insects and mites around 2500 BC (Dent, 2000). Since then to meet the growing population chemical use against major insect pests has been in increasing trend. Soybean farmers too usually spray insecticide to control *R. pedestris* because of its convenience and quick action (Lee *et al.,* 2004) found that Fenitrothion was highly susceptible to developmental stages of *R. pedestris*. Diflubenzuron significantly reduced not only the adult longevity and the number of eggs laid per female but also adversely affected the period before oviposition and ovarian development of *R. pedestris* (Kim *et al.,* 1992). Bae *et al.,* 2005) reported susceptibility of seven insecticides for five stink bugs species including *R. pedestris*. However, stink bugs are difficult to control due to its high mobility (Choi *et al.,* 2005; Wada *et al.,* 2006).

Trap crops

The principle of trap crops is that virtually all pests show a distinct preference to certain plant species, cultivars, or a certain crop stage (Newsom and Herzog, 1977). Mcpherson and Newsom (1984) reported 70-85% of all *N. viridula* in soybean crops were attracted to trap crop strips covering only 1-10% of the total cropping area. Release of *Trissolcus basalis* into early matures soybean trap crops as soon as *N. viridula* start colonizing fields may constitute an important tactic in stink bug management (Correa –Ferreira and Moscardi; 1996). Youn and Jung (2008) suggested use of trap cropping for the enhancement of biological control agent of *R. pedestris.*

Aggregation pheromone trap

Males of the bean bug, *R. pedestris*, an economically important soybean pest, emit an aggregation pheromone that attracts conspecific nymph and adults (Leal *et al.,* 1995; Mizutani *et al.,* 1997; Huh and Park, 2005; Son *et al.,* 2009). Masuta *et al.,* (2001) reported the number of *R. pedestris* adults caught in the pheromone traps increased with increasing amounts of the pheromone lures up to 100mg. The most effective pheromone trap was fish trap type installed 60 cm above ground (Paik *et al.,* 2009a).

2.4 *Ooencyrtus* sp.

Ooencurtus sp. is an egg parasitoid of several phytophagous bugs in Bangladesh and the parasitoid is gregarious in a large host, such as *R. pedestris.* On encountering a potential host, egg parasitoids examine its surface with antennae while walking over it. After antennal examination they begin to penetrate the host eggs. Host recognition by egg parasitoids is dependent mainly on physical and chemical factors on the hosts surface (Schmidt, 1994). *Ooencyrtus nezarae* parasitized the eggs of 13 hemipteran species including the pentatomid soybean pests' *Piezodorus hybneri* Gmelin, *Dolycoris baccarum* Linnaeus, and *Nezara antennata* Scott (Takasu and Hirose, 1985; Hirose *et al.,* 1996; Mizutani *et al.,* 1996; Zhang *et al.,* 2005). Fresh as well as refrigerated eggs of *R. pedestris* were evaluated for the effect on parasitism by *Ooencyrtus* sp. and found to be suitable for the parasitoid (Alim, 2019 unpublished).

Taxonomic position of *Ooencyrtus* sp.

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Hymenoptera

Family: Encyrtidae

Genus: *Ooencyrtus*

Species: Trying to identify

CHAPTER III

MATERIALS AND METHODS

The experiment was conducted in the laboratory of the Department of Entomology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur during the period from June, 2018 to July, 2019. Details materials and methods were used during the study period are described below.

3.1 Rearing of *R. pedestris*

Adults of *R. pedestris* were collected from infested country bean fields in HSTU campus in June, 2018 and maintained in the laboratory according to the method described by Alim and Lim (2009). The nymph and adults were kept in acryl cages (40L × 40W × 40H cm) each with windows in three lateral sides covered with meshed screens for ventilation at ambient temperature 31.62°C ± 0.16, relative moisture 79.25% ± 1.60 and natural photoperiod. Temperature and relative humidity were measured by the Hydro-thermometer (HTC-1), China. Ascorbic acid dissolved in water and soybean seeds were provided to adult *R.*

pedestris in insect breeding dishes. Nymphs were led with soybean seeds and bean plants with cotyledonous leaves. Four pieces of gauze were placed in the top and bottom corners inside the adult cages as oviposition substrates. Eggs on the gauze were collected and new gauze was placed in the acryl cages daily. The cage was cleaned two times a week.

Plate 3.1: Rearing of *R. pedestris*

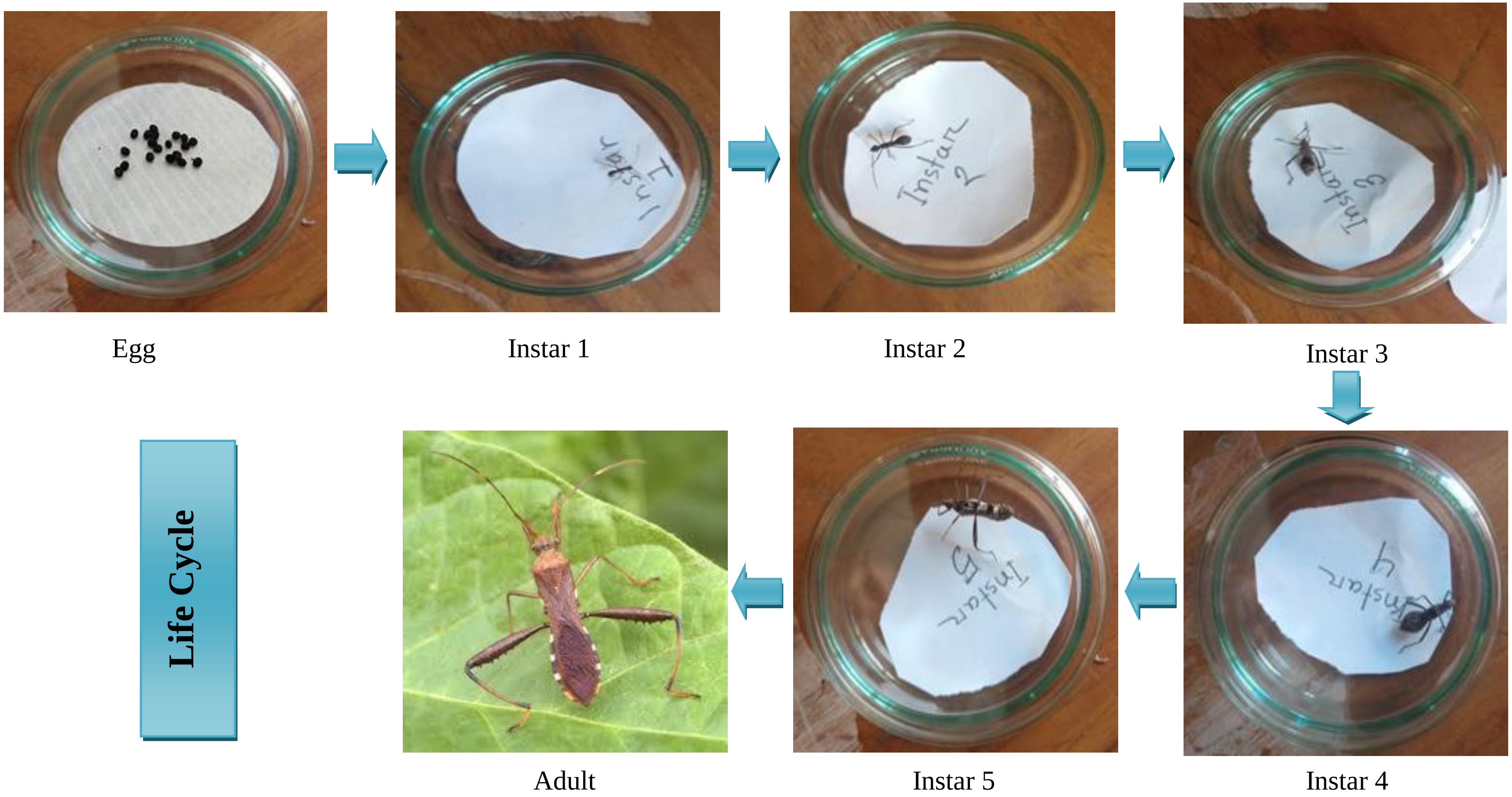

Plate 3.2: Life Cycle of Bean bug (*R. pedestris*)

3.2 Rearing of *Ooencyrtus* sp.

Ooencyrtus sp. were collected from parasitized natural eggs of *R. pedestris* from country bean field and maintained on in the laboratory at ambient temperature 31.62°C ± 0.16, relative moisture 79.25% ± 1.60 and natural photoperiod. *Ooencyrtus* sp. males and females were placed in a plastic centrifugal tube (50 ml) (SP Life Sciences Co. Ltd, Korea) and provided with honey on the surface and a piece of moistened cotton on the bottom of the tube. Water and honey were supplied at three days interval.

Plate 3.3: Rearing of *Ooencyrtus* sp.

Plate 3.4. Female and male of *Ooencyrtus* sp.

3.3 Seasonal abundance of *R. pedestris* and its egg parasitoids on different locations

To determine the parasitism rate of natural eggs, 10 country bean plants were randomly selected and examined to collect eggs of *R. pedestris* every 10 days from August to November 2018 at each site. The number of nymphs and adults of *R. pedestris* was also recorded while examining country bean plants to collect *R. pedestris* eggs. Eggs were brought to the laboratory and placed in micro tubes (2 ml capacity) individually and kept in ambient temperature. After hatching the eggs simultaneously both *R. pedestris* and its parasitoids were found and recorded daily. Families of emerged parasitoids were determined according to Goulet and Huber (1993), and species were identified according to the keys of Tachikawa (1963), Mineo (1981), and Zhang *et al.*, (2005). The secondary sex ratio of the emerged parasitoids was recorded. Eggs that produced neither a host nymph nor an adult parasitoid were dissected under a stereomicroscope. Eggs were considered as parasitized if they contained dead immature parasitoids.

3.4 Monitoring of *R. pedestris* and its egg parasitoids by pheromone traps

Aggregation pheromone traps were made by iron frame and covered with mosquito net (32 L×13 D cm) containing aggregation pheromone lures inside (Green Agro-Tech Co. Ltd., Kyungsan, Korea, 50 micro-liters / lures). [Myristyl isobutyrate, *(E)*-2-hexenyl *(E)*-2-hexenoate and *(E)*-2-hexenyl *(Z)*-3-hexenoate at the ratio of 1:5:1] Both the opening of pheromone trap allows *R. pedestris* to enter. The pheromone trap was placed in the country bean canopy by attaching with thread and bamboo pole. Inside the trap a thread was used for laying eggs by *R. pedestris* adults. Each location installed one pheromone trap. Adults and nymph of *R. pedestris* were counted daily and egg lays by adult *R. pedestris* were collected and brought into the laboratory and placed in micro tubes (2 ml capacity) individually and kept in ambient temperature. Hatching of host eggs and the emergence of adult parasitoids were recorded daily. Parasitoids were identified above mentioned procedure.

Plate 3.5: Pheromone lures

Plate 3.6: Monitoring of *R. pedestris* and its egg parasitoids by pheromone traps

3.5 Effects of host egg age on host acceptance behavior by *Ooencyrtus* sp.

After 0, 24, 48, 72 and 96 hours of lay, the eggs of each time period were taken and placed in the insect breeding Petri dish. One mated, inexperienced, 4 days old female was introduced. Host acceptance behaviors were observed under a stereomicroscope after categorizing them as drumming, drilling, host feeding and oviposition according to Strand and Vinson (1983), and Noda (1993). Time spent on each event was recorded for each of the eggs when the parasitoid displayed complete host acceptance behavior. These procedures were repeated with 10 female parasitoids.

Plate 3.7: Host acceptance by *Ooencyrtus* sp. female

3.6 Effect of host eggs on the biological attributes of *Ooencyrtus* sp.

After 0, 24, 48, 72 and 96 hours of laid eggs, ten eggs of *R. pedestris* were placed in an insect breeding dish (4 H×10 D cm). A 4-day old, mated, new *Ooencyrtus* sp. female was allowed to parasitize the eggs for 24 hours at 31.62°C ± 0.16, 79.25% ± 1.60. After 24 hours the female *Ooencyrtus* sp. were withdraw and the eggs were then placed individually in micro tubes (2ml capacity) with the honey drop. The emergence of parasitoids from each egg was observed daily. The number of host eggs parasitized, adult emergence, sex ratio, and development time of male and female of *Ooencyrtus* sp. were observed. Host eggs containing parasitoids that failed to develop or to emerge were also considered as parasitized from dissection of each egg. These procedures were replicated ten times for each age period.

The parasitoid was collected and placed in a parasitoid rearing tube (50 ml capacity). Fresh honey and moistened cotton were provided every three days, and the parasitoids were transferred to new tubes as needed. To find the effect of time on the size of the emerged parasitoids, a total of 30 both males and females of each hour were randomly selected in each group and their hind tibia lengths were measured under Stereo microscope (Am Scope, China) using a micrometer.

3.7 Survival of *R. pedestris* refrigerated eggs

Eggs of *R. pedestris* were kept in the refrigerator for 0, 5, 10, 15, 20, 25 and 30 days. After refrigeration, 50 host eggs from each refrigeration period was removed to an insect breeding Petri dish (4 H×10 D cm) and were kept in laboratory conditions. Survival was measured as the number of eggs hatch divided by the total number of eggs tested.

3.8 Effect of refrigerated host eggs on the biological parameters of *Ooencyrtus* sp.

After 0, 15, 30, 60 and 120 days of refrigeration, 10 eggs on each time period were removed from the refrigerator and placed in an insect breeding dish. A 4-day old, mated, new *Ooencyrtus* sp. female was allowed to parasitize the eggs for 24 hours. The eggs were then placed individually in micro tubes (2 mi capacity). The emergence of parasitoids from each egg was observed daily. The number of host eggs parasitized, emergence rate, sex ratio, and development time of male and female *Ooencyrtus* sp. was measured. Host eggs containing parasitoids that failed to develop or to emerge were also considered as parasitized from dissection of each egg. These procedures were replicated ten times for each storage period.

The parasitoid was collected and placed in a parasitoid rearing tube. Fresh honey and moistened cotton was provided every three days, and the parasitoids were transferred to new tubes as needed and used for rearing.

3.9 Statistical analyses

Emergence rate, adult sex ratio, survival percent and seasonal parasitism rate data were subjected to a chi square test of a contingency table and a Tukey- type multiple comparison test for post hoc analysis (Zar, 2010). Number of parasitized host egg, number of parasitoids emerged, development time, hind tibia length of parasitoids, unparasitized egg or nymphs and adults recorded in the field were analyzed by a Kruskal- Wallis single factor analysis of variance by rank. If significance was detected, multiple comparisons were conducted using the Duncan test (Zar, 2010).

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Seasonal abundance of *R. pedestris* and its egg parasitoids on different locations

Eggs, nymphs and adults of *R. pedestris* were found throughout the sampling period. The seasonal number of eggs (H_C = 16.557, df = 2, P < 0.001), nymphs (H_C = 1.655, df = 2, P = 0.437) and adults (Hc = 1.387, df = 2, P = 0.500) of *R. pedestris* was not significantly different among the locations (Fig.1& 2). The seasonal total parasitism rate from natural host eggs at each sampling site was separated by parasitoid species, i.e., gregarious parasitoids *Ooencyrtus* sp. and solitary parasitoid *Gryon nigricorne*. From the statistical analysis throughout the season, the total parasitism rate by both species were found to be higher in Mohabolipur compared to Noshipur and HSTU Campus field (χ^2 = 16.72, df = 2, P < 0.001) (Fig.2). Parasitism rate by *G. nigricorne* on natural host eggs was found higher in Mohabolipur compared to HSTU campus field and Noshipur (χ^2 = 130.03, df = 2, P < 0.001) On the other hand, the parasitism rate of gregarious *Ooencyrtus* sp. (χ^2 = 46.963, df = 2, P < 0.001) found higher in Noshipur among three study sites (Fig.3). In this study higher parasitism by *Ooencyrtus* sp. was observed in August i.e., is the foliar stages of country bean plant, whereas *G. nigricorne* parasitized more eggs in November i.e., pod formation stages in Bangladesh. Noda (1993) and Takasu *et al.*, (1998) found higher parasitism on leaves than pods by *O. nezarae*, whereas *G. japonicum* parasitized more hosts on pods than on leaves. Mizutani (1994) found that *G. japonicum* tended to be a successful competitor when the parasitoids attacked the hosts a short time after an attack by *O. nezarae*.

4.2 Monitoring of *R. pedestris* and its egg parasitoids by pheromone traps

Throughout the sampling period eggs, nymphs and adults of *R. pedestris* was found in the pheromone traps in the different study sites. Neither the seasonal number of unparasitized eggs (H_C = 2.170, df = 2, P = 0.338) nor nymphs (H_C = 0.225, df = 2, P = 0.880) and adults

(Hc = 0.748, df = 2, P = 0.688) of *R. pedestris* was significantly different among the study sites (Fig. 4). However, unparasitized eggs, adults and nymph observed higher in HSTU campus. From the statistical analysis throughout the season, the total parasitism rate was not significantly different but found higher in HSTU campus compared to Noshipur and Mohabolipur field (χ^2= 2.074, df = 2, P = 0.355) (Fig.5). The seasonal total parasitism rate on natural host eggs at each sampling site was separated by parasitoid species, i.e., gregarious parasitoids *Ooencyrtus* sp. and solitary parasitoid *Gryon nigricorne.* Parasitism rate of both *Ooencyrtus* sp. (χ^2 = 29.85, df =2, P < 0.001) and *G. nigricorne* (χ^2 = 44.963, df = 2, P < 0.001) on natural host eggs was found higher in HSTU campus compared to other study sites (Fig.6). In this study, higher parasitism by *Ooencyrtus* sp. was found higher in August i.e., is the foliar stages of country bean plant, whereas *G. nigricorne* parasitized more eggs in November i.e., pod formation stages in Bangladesh. Noda (1993) and Takasu *et al.,* (1998) found higher parasitism on leaves than pods by *O. nezarae*, whereas *G. japonicum* parasitized more hosts on pods than on leaves.

4.3 Effects of host egg age on host acceptance behavior by *Ooencyrtus* sp.

All *Ooencyrtus* sp. showed complete processes of host acceptance behaviors on the eggs of the host *R. pedestris*. Behaviors showed by *Ooencyrtus* sp. could be divided into Drumming, drilling, feeding and ovipositing. Each phase consisted of one or more bouts. The duration of host acceptance behaviors displayed by the egg parasitoid is shown in Table 1. Drumming was characterized by moving antennae up and down over the exposed surface of the eggs, the female remaining still or standing over the eggs. The duration of drumming decreased as the age of the host egg increased (H_C = 25.64, df = 4, P < 0.001). The eggs of *R. pedestris* of 0 hour (Control) age showed the longest duration of drumming by the parasitoid. The female parasitoid elevated her body after drumming and lowered her abdomen to tap the lateral surface of the host egg with the tip of her ovipositor. *Ooencyrtus* sp. inserted ovipositor after few seconds of tapping. The sheath was removed from ovipositor during insertion and the abdomen return to normal position. Ovipositor continuously rotated by the female for penetration of the host chorion and suddenly touched the point where her ovipositor entered by lowering her body. Touching continued for several seconds before she raised her abdomen again. The action of touching repeated several times. The mean time spent on drilling is decrease with the rises of host eggs age compared to fresh eggs (0 hour) (H_C = 34.62, df = 4, P < 0.001). After drilling, the female parasitoid reinserted her ovipositor into the host and moved it up and down several times, resulting in a drop of translucent liquid forming at the point of ovipositor insertion. After removing and sheathing her ovipositor, she backed away and lapped up the drop as well as again inserted ovipositor into the host. Then she inserted her ovipositor to laid eggs and withdrew the ovipositor. After completing oviposition, the parasitoid drummed the host and finally descended from the host. The mean time spent on oviposition & feeding is decreased with the age of the host egg increase (H_C = 39.72, df = 4, P < 0.001) compared to 0 hour. This decrease is probably caused by the decreasing amount of host content that female can extract. *Ooencyrtus* sp. female fed on host fluid such as yolk or hemolymph that exudes from punctures of hosts after the pumping action of the ovipositor. Newly deposited *R. pedestris* eggs consist almost entirely of yolk, but as the host embryo develops the amount of yolk decreases. Some gregarious egg parasitoids are known to allocate eggs they lay in a host based on host age (Lewis & Bedlinger 1969, Marston & Ertle 1996). In this study, 0-hour eggs required more time for oviposition compared to 96 hours.

4.4 Effect of host eggs on the biological attributes of *Ooencyrtus* sp.

The number of eggs parasitized by *Ooencyrtus* sp. was influenced by the different host egg ages. *Ooencyrtus* sp. can successfully parasitize host eggs up to 48 hours without a significant reduction in parasitism. However, for the host egg of 96 hour the parasitized host eggs decreased approximately 29% (H_C = 21.338, df = 4, P < 0.001) compared to the control

(Table 2). The emergence rate and the sex ratio (χ^2=11.07 *df* = 4, *P* = 0.242) of *Ooencyrtus* sp. were unaffected by host egg ages (Table 2). The sex ratios of the emerged parasitoids were all female-biased without statistical significance among the different time periods. However, the total number of parasitoids emerged that from parasitized eggs significantly decreased with the rise of host egg age (H_C = 23.897, *df* = 4, *P* < 0.001). Mean development time (from eggs to adult) of *Ooencyrtus* sp. male and female increased significantly as host eggs aged increased (male H_C = 87.056, *df* = 4, *P* < 0.001; Female H_C = 193.125, *df* = 4, *P* < 0.001) (fig.7). In this study, the highest mean development time found in 96 hours of age compared to control (0 hour). The low nutritional quality attributed to older host eggs can slow down offspring development rate (Godfray, 1994). Hind tibia length of *Ooencyrtus* sp. decreased as the age of the host eggs increased (*Hc* = 35.390, *df* = 4, *P* < 0.001) (Fig. 8). The significant changes in the number of parasitized host eggs, the total number of parasitoid and development time might be caused by the decrease in the nutritional quality of eggs with the increase of egg aged (Alim and Lim, 2010).

4.5 Survival of refrigerated *R. pedestris* eggs

The percentage of *R. pedestris* eggs hatched was significantly influenced by the length of refrigeration time (χ^2= 87.102, *df* = 6, *P* < 0.001). Survival dropped from 99% from the fresh eggs to 7% from the eggs refrigerated for 25 days. In this study, no eggs hatched after 30 days of refrigeration due to loss of viability (Fig. 9). Alim and Lim (2009) found that *R. pedestris* eggs refrigerated for 30 days become non-viable. Chen and Leopold (2007) killed eggs of *Homalodisca coagulate* say by 5 days at 2^0 C chilling, and Mohmoud and Lim (2007) found that eggs of *Dolycoris baccarum* L. do not hatch after 20 days of refrigeration at 2.1^0C.

4.6 Effect of refrigerated host eggs on the biological attributes of *Ooencyrtus* sp.

The number of eggs parasitized by *Ooencyrtus* sp. was influenced by the different refrigeration times. *Ooencyrtus* sp. can successfully parasitize host eggs up to 60 days without a significant reduction in parasitism. However, for the host egg of 120-day refrigeration time period, the parasitized host eggs decreased approximately 50 % (H_C = 14.93, *df* = 4, *P* = 0.005) compared to the control (Table 3). The emergence rate and the sex ratio (χ^2= 10.06, *df* = 4, *P* = 0.282) of *Ooencyrtus* sp. were unaffected by host egg ages (Table 3). The sex ratios of the emerged parasitoids were all female biased without statistical significance among the different time periods. However, total number of parasitoids that emerged from parasitized eggs significantly decreased with the rises of refrigeration periods (H_C = 39.299, *df* = 4, *P* < 0.001). Mean development time (from eggs to adult) of *Ooencyrtus* sp. male and female increased significantly as host eggs aged increased (male H_c= 107.88, *df* = 4, *P* < 0.001; Female H_C = 234.10, *df* = 4, *P* < 0.001) (Fig. 10). In addition, less development time find in control (0 hour) whereas 120 days of refrigeration showed the highest mean time for development. Leopold (1998) reported refrigeration might affect the physiological process of host egg that is deleterious for the development of parasitoid. The significant changes in the number of parasitized host eggs, total number of parasitoid and development time might be caused by the decrease in the nutritional quality of eggs refrigerated for longer periods (Kivan and Kilic, 2005; Chen and Leopold, 2007; Alim and Lim 2009; Alim and Lim 2010). In this study, refrigeration might inhibit a physiological change or reduces nutritional quality in a host egg that is detrimental to the development of the parasitoid.

CHAPTER V

SUMMARY AND CONCLUSION

Riptortus pedestris is a newly invasive pest of bean field in Bangladesh. Seasonal abundance of *R. pedestris* and its eggs parasitoids were investigated during August to November 2018. Eggs, nymphs and adults of *R. pedestris* were observed in three study locations. *R. pedestris* were captured by aggregation pheromone traps during the study period in three different locations. *Ooencyrtus* sp. and *Gryon nigricorne* were parasitized the *R. pedestris* natural eggs in field conditions. *Ooencyrtus* sp. was observed in the month of August to September when the bean plants were vegetative stages. *Gryon nigricorne* was observed in the pod formation stages of bean. Seasonal occurrences of two egg parasitoids were monitoring by aggregation pheromone traps as well as enhance the field parasitism. This study indicates that *Ooencyrtus* sp. can successfully parasitized *R. pedestris* eggs up to 48 hour and 30 days in fresh and refrigerated host eggs, respectively without significant reduction in parasitism. In addition, fresh and refrigerated host eggs have no significant effect on emergence rate and sex ratio. Fresh host eggs age can not affect the host acceptance behaviors of *Ooencyrtus* sp up to 48 hours. Biological attributes of emerged parasitoids were also not affected by refrigeration of host eggs compared to fresh eggs. Therefore, *Ooencyrtus* sp. can successfully parasitized fresh eggs up to 48 hours and refrigeration of *R. pedestris* eggs can be an alternative method to mass rear of *Ooecyrtus* sp., one of the major gregarious egg parasitoid.

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Table 1: Duration (min ± SE) of host acceptance behaviors displayed by *Ooencyrtus* sp. on fresh eggs of *R. pedestris*

Behaviors	Age of host eggs (hours)				
	0 (Control)	24	48	72	96
Drumming	2.46 ± 0.13 a	2.06 ± 0.06 b	1.97 ± 0.04 b	1.89 ±0.08 b	1.18 ± 0.04 c
Drilling	14.31 ± 0.19 a	14.25 ± 0.28 a	13.82 ± 0.31 a	11.99 ± 0.25 b	10.88 ± 0.37 c
Feeding & Oviposition	35.42 ± 0.44 a	34.23 ± 0.66 ab	32.61 ± 0.56 b	27.68 ± 0.77 c	24.09 ± 0.44 d

Numbers in each row followed by the same letter are not significantly different (P < 0.05).

See text for statistics

Table 2: Effect of host eggs age on the biological attributes of *Ooencyrtus* sp.

	Age of the host eggs (hours)				
	0 (control)	24	48	72	96
Number of parasitized eggs /females ± SE	7.6 ± 0.26 a	7.4 ± 0.26 a	6.9 ± 0.40 a	5.4 ± 0.50 b	4.7 ± 0.52 b
Proportion of male ±SE	0.20 (42/217) a	0.19 (42/219) a	0.25 (47/191) a	0.24 (36/151) a	0.20 (28/138) a
Emergence rate	1.00 (217/217) a	1.00 (219/219) a	1.00 (191/191) a	1.00 (151/151) a	1.00 (138/138) a
Total number of parasitoids ± SE	22 ± 0.76 a	21.7 ± 0.83 a	22.2 ± 1.00 a	15.1 ± 1.13 b	14 ± 1.56 b

Numbers in each row followed by the same letter are not significantly different ($P < 0.05$).

See text for statistics.

Table: 3. Effect of refrigerated eggs on biological attributes of *Ooencyrtus* sp.

	Duration of Refrigeration (Days)				
	0 (control)	15	30	60	120
Number of parasitized eggs /female ± SE	7.30 ± 0.30 a	7.20 ± 0.30 a	6.80 ± 0.33 a	5.20 ± 0.29 b	2.20 ± 0.36 c
Proportion of male ±SE	0.20 (42/215) a	0.33 (52/159) a	0.32 (42/127) a	0.29 (28/97) a	0.33 (24/71) a
Emergence rate	1.00 (215/215) a	1.00 (159/159) a	1.00 (127/127) a	1.00 (97/97) a	1.00 (71/71) a
Total number of parasitoids ±SE	21.50 ± 0.78 a	15.90 ± 0.87 b	12.60 ± 0.83 c	9.70 ± 0.66 d	7.50 ± 0.82 d

Numbers in each row followed by the same letter are not significantly different ($P < 0.05$).

See text for statistics

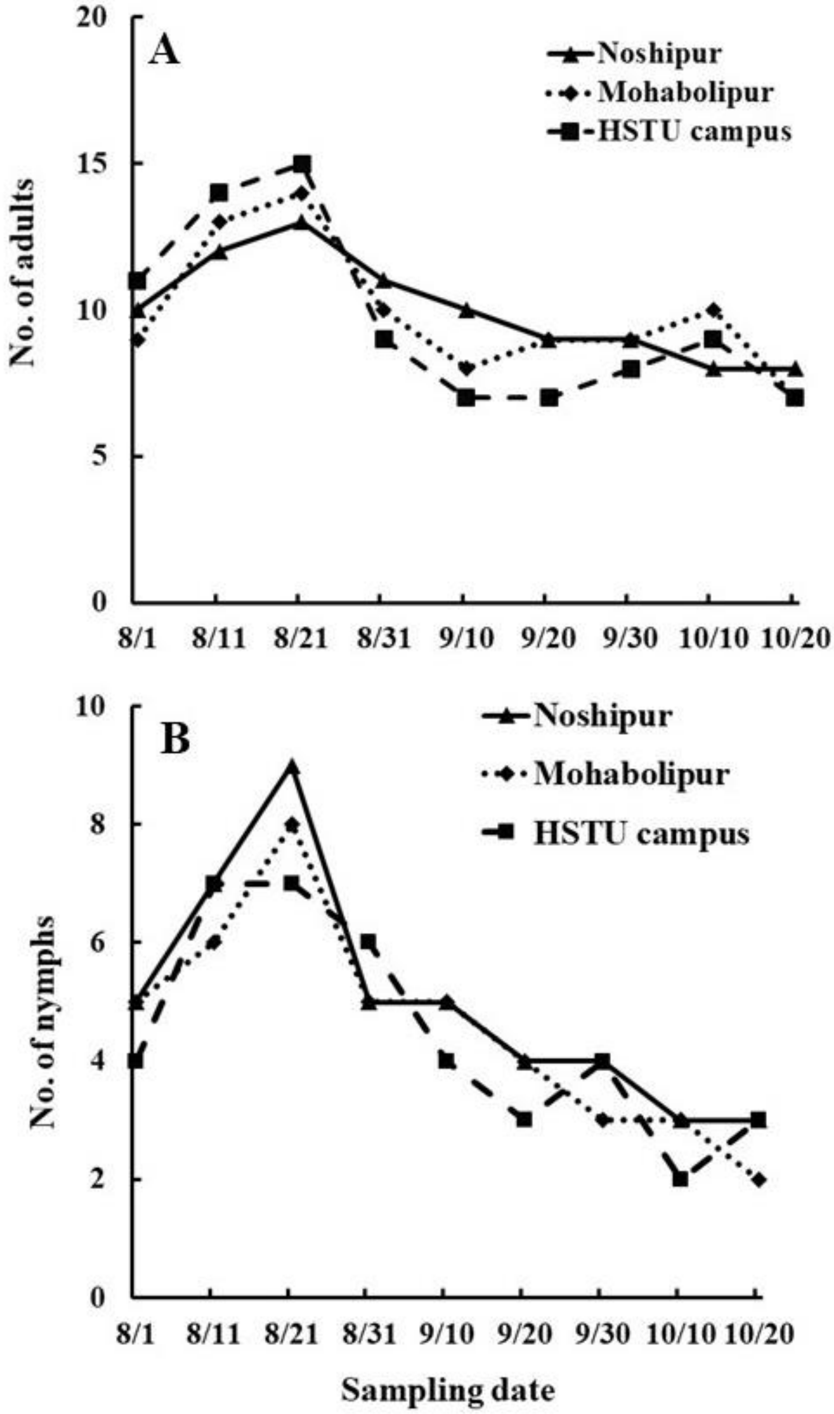

Figure 1. Number of Adults and nymphs of *R. pedestris* collected by monitoring from three different study sites. Analysis were conducted on the seasonal data using Kruskal-Wallis single factor analysis of variance by rank.

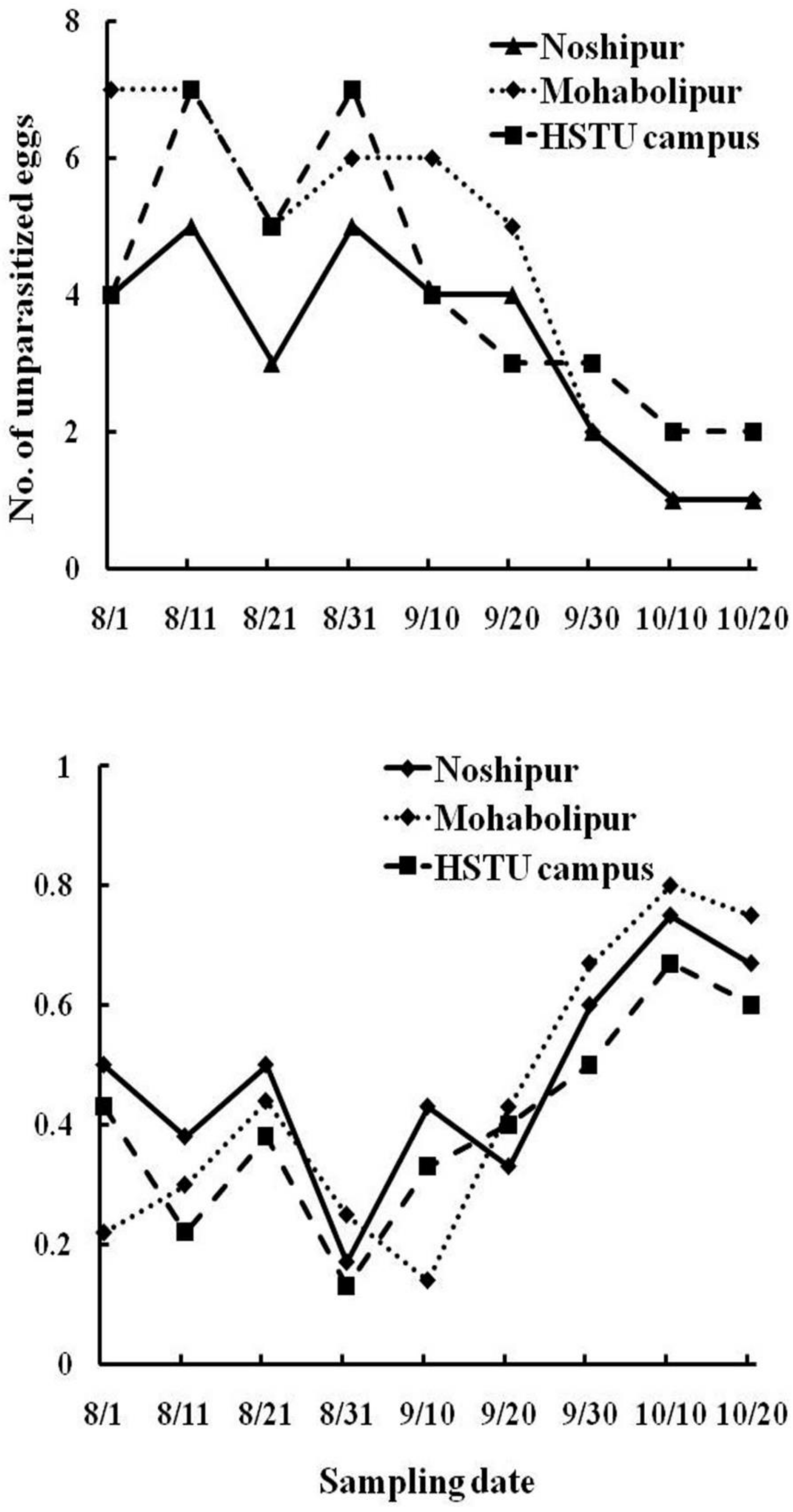

Figure 2. Number of *Riptortus pedestris* eggs collected by monitoring from three different study sites. Analysis were conducted on the seasonal data using Kruskal-Wallis single factor analysis of variance by rank.

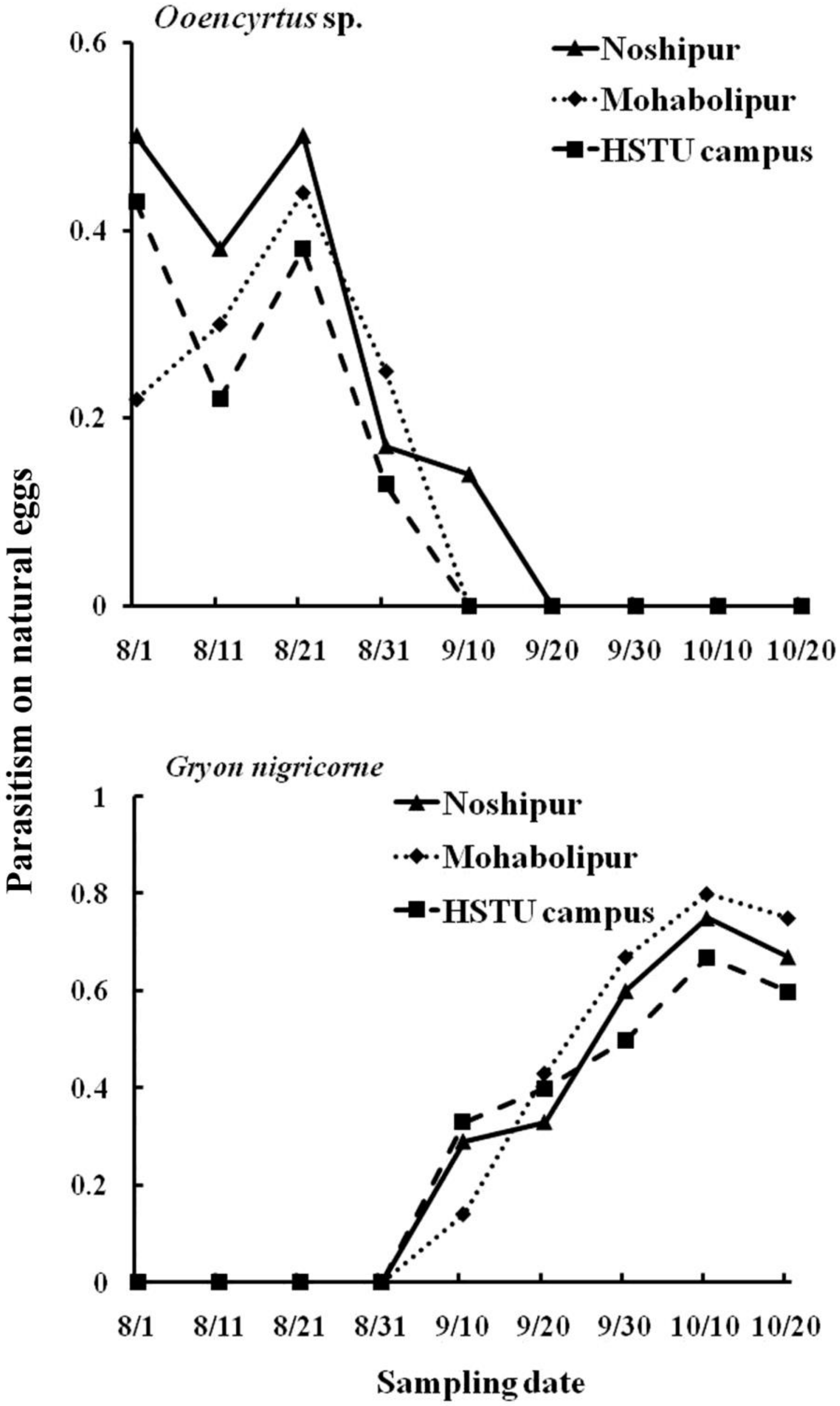

Figure 3. Seasonal parasitism rate by *Ooencyrtus* sp. and *G. nigricorne* on natural eggs collect by monitoring from three different study sites.

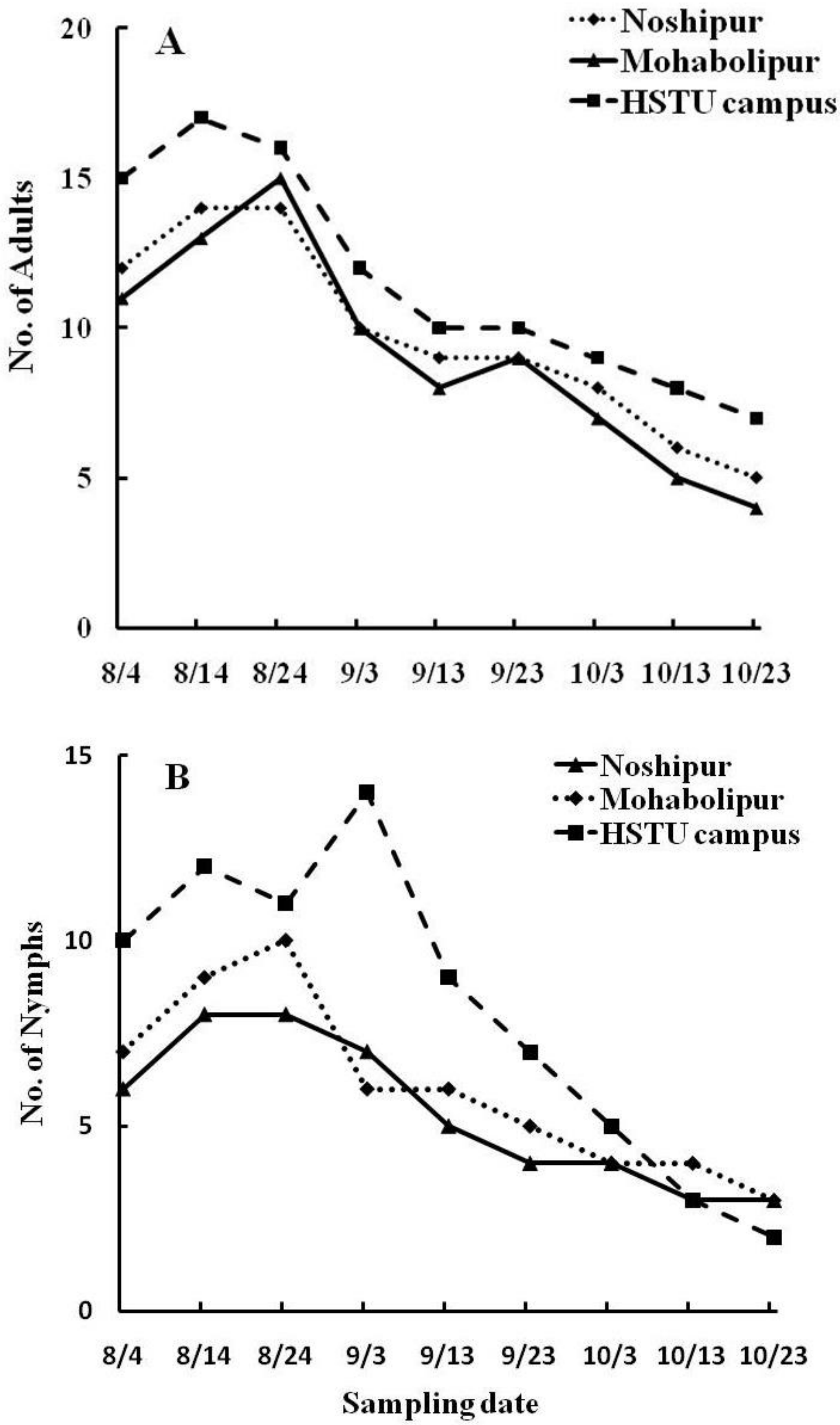

Figure 4. Number of adults and nymph of *R. pedestris* collected from aggregation pheromone traps from three different study sites. Analysis were conducted on the seasonal data using Kruskal-Wallis single factor analysis of variance by rank.

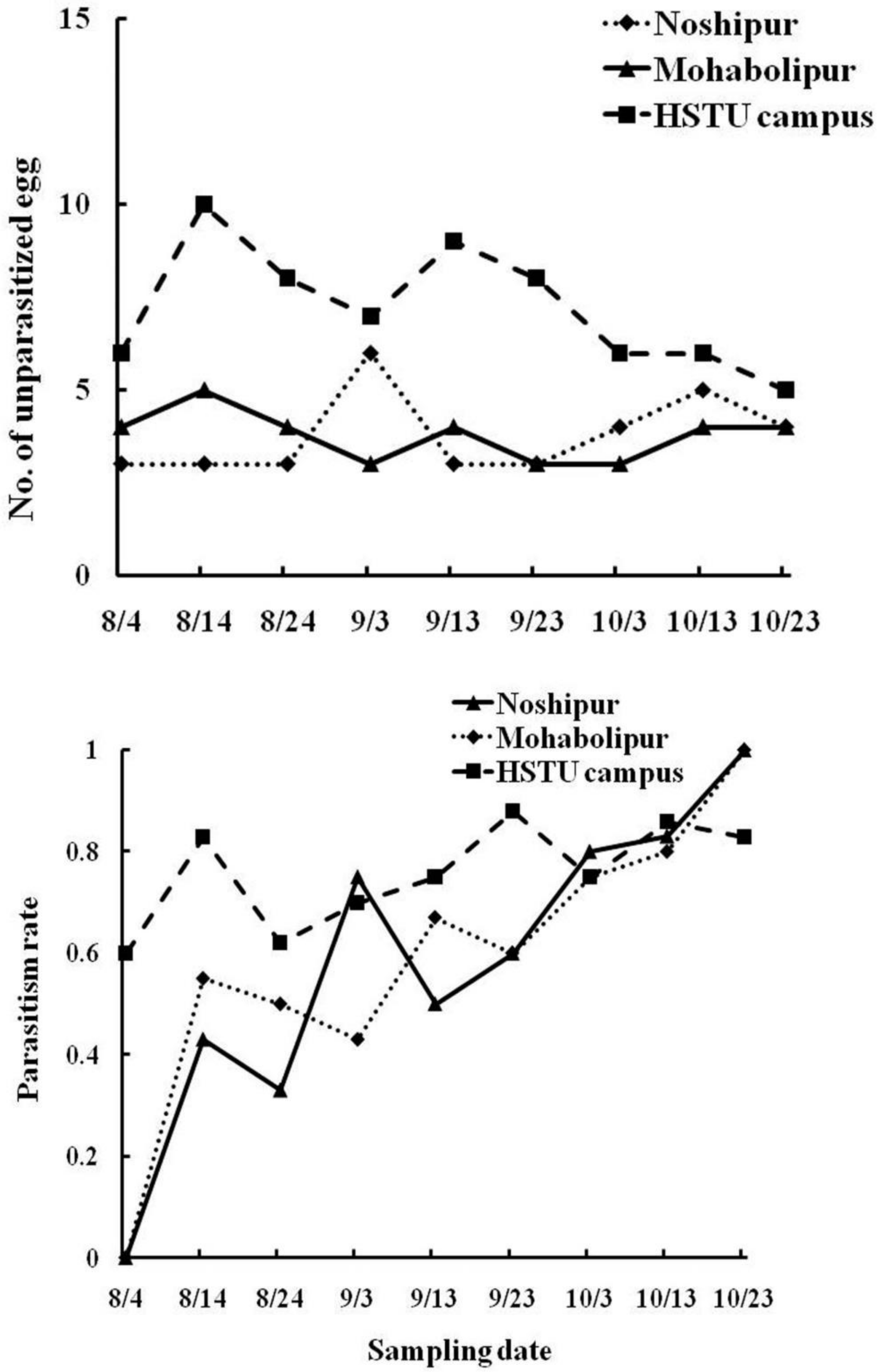

Figure 5. Number of *Riptortus pedestris* eggs collected from aggregation pheromone traps from three different study sites. Analysis were conducted on the seasonal data using Kruskal-Wallis single factor analysis of variance by rank.

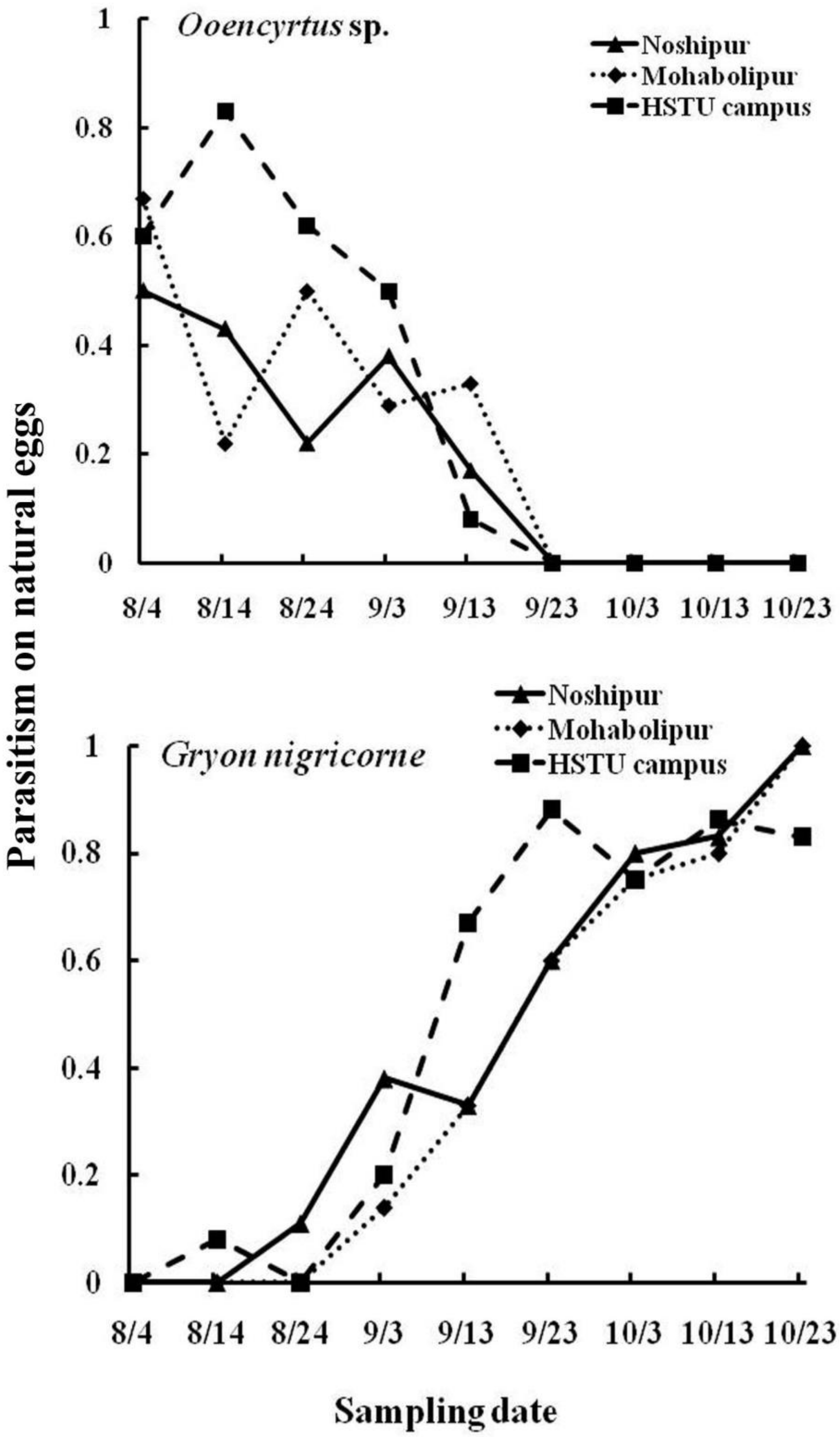

Figure 6. Seasonal parasitism rate by *Ooencyrtus* sp. and *G. nigricorne* on natural eggs collected from three different study sites from aggregation pheromone traps.

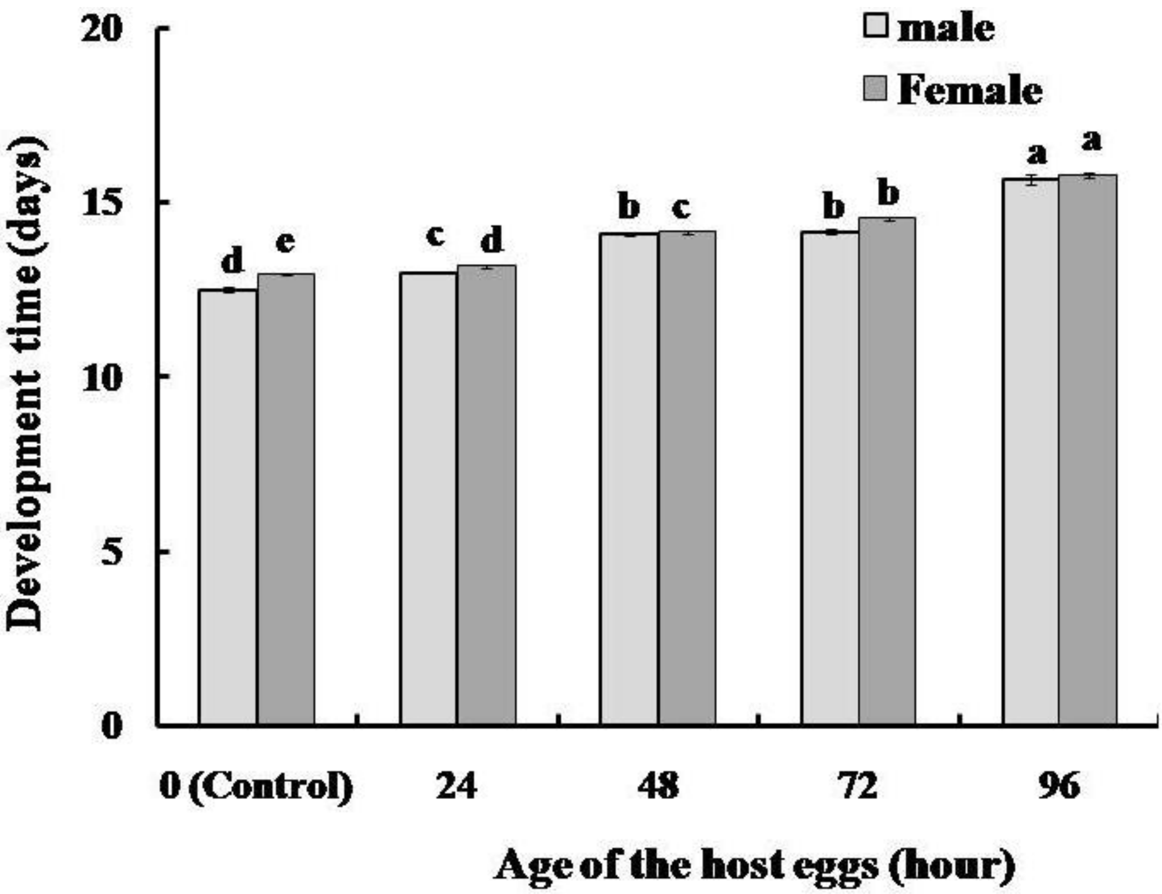

Figure 7. Development time (± SE) of *Ooencyrtus* sp. (male & female) developed in fresh egg of *R. pedestris*. Values followed by different letters are significantly different by Kruskal-Wallis single factor analysis of variance by rank ($P < 0.05$).

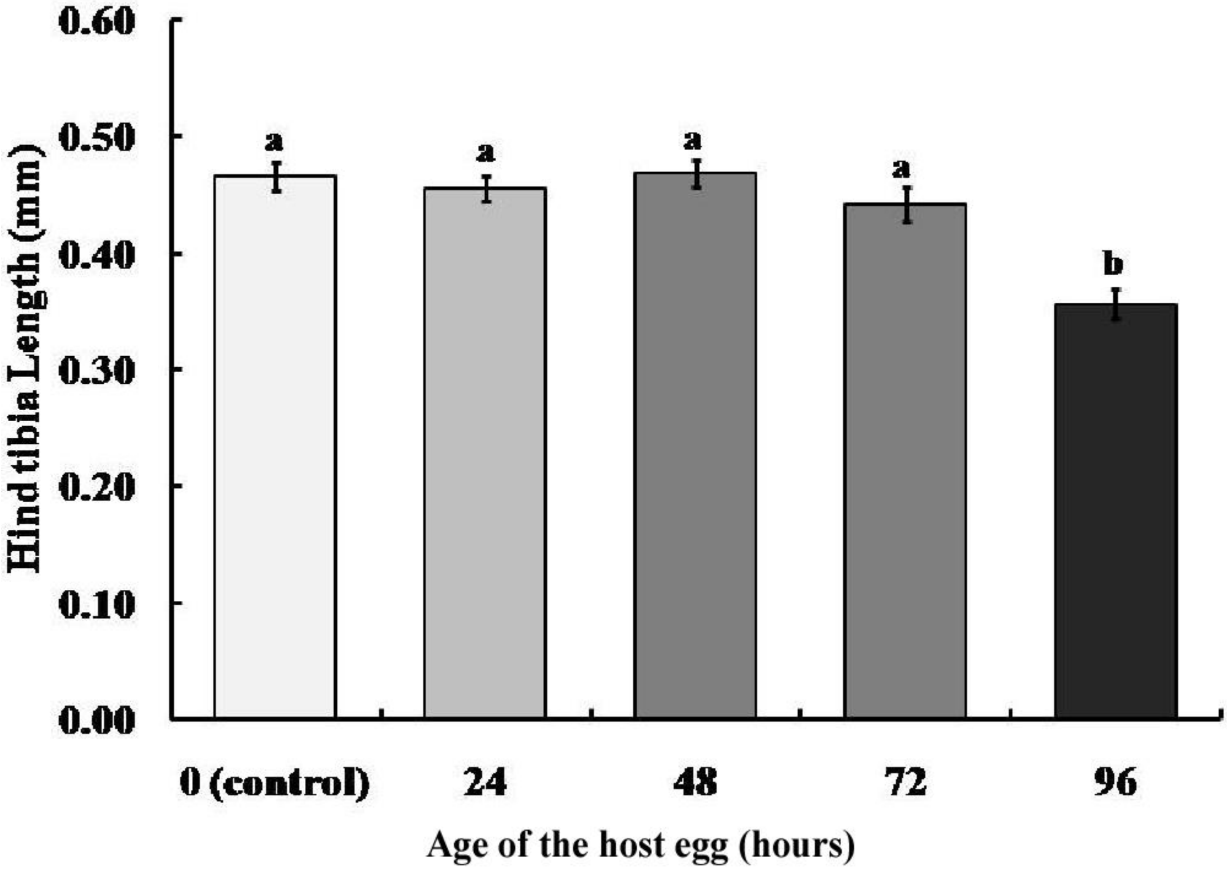

Figure 8. Hind tibia length (± SE) of *Ooencyrtus* sp. reared on *R. pedestris* fresh eggs. Values followed by different letters are significantly different by Kruskal-Wallis single factor analysis of variance by rank ($P < 0.05$).

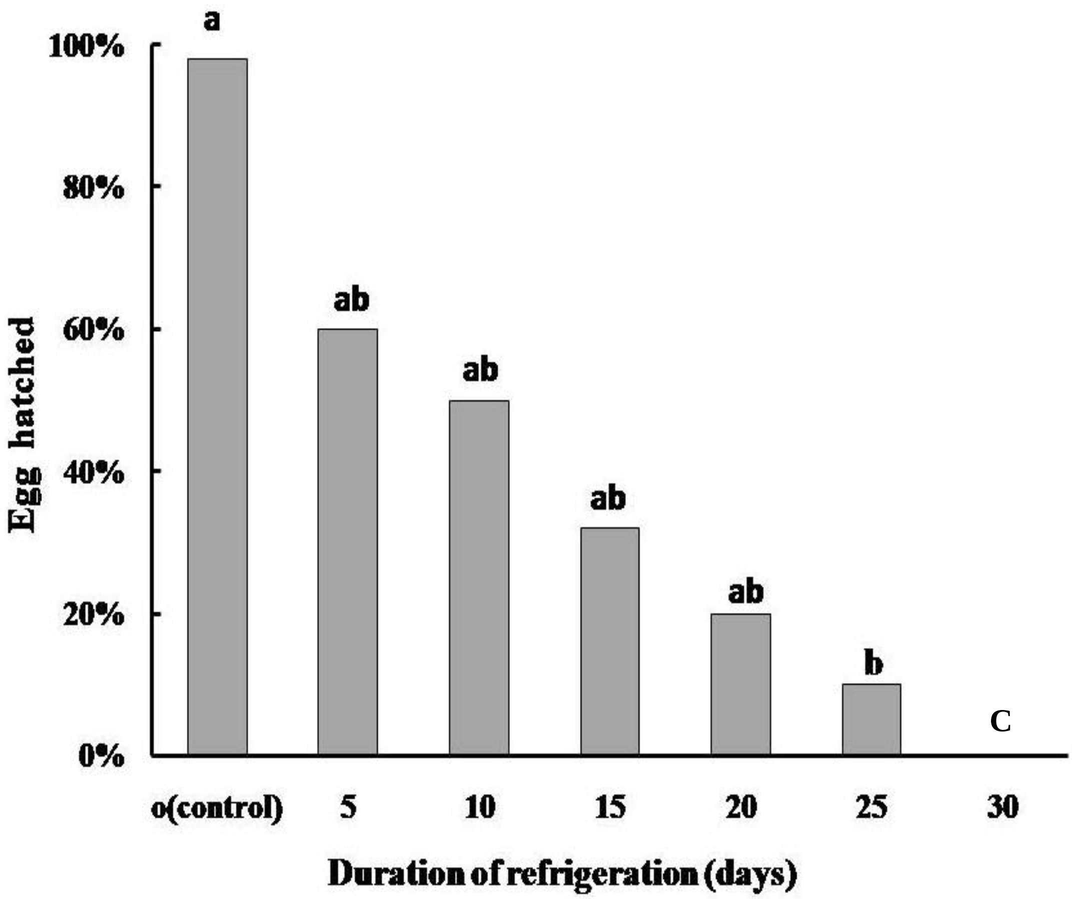

Figure 9. Survival of refrigerated *R. pedestris* eggs. Values followed by a different letter are significantly different from multiple Comparison tests analogous to Tukey test ($P < 0.05$).

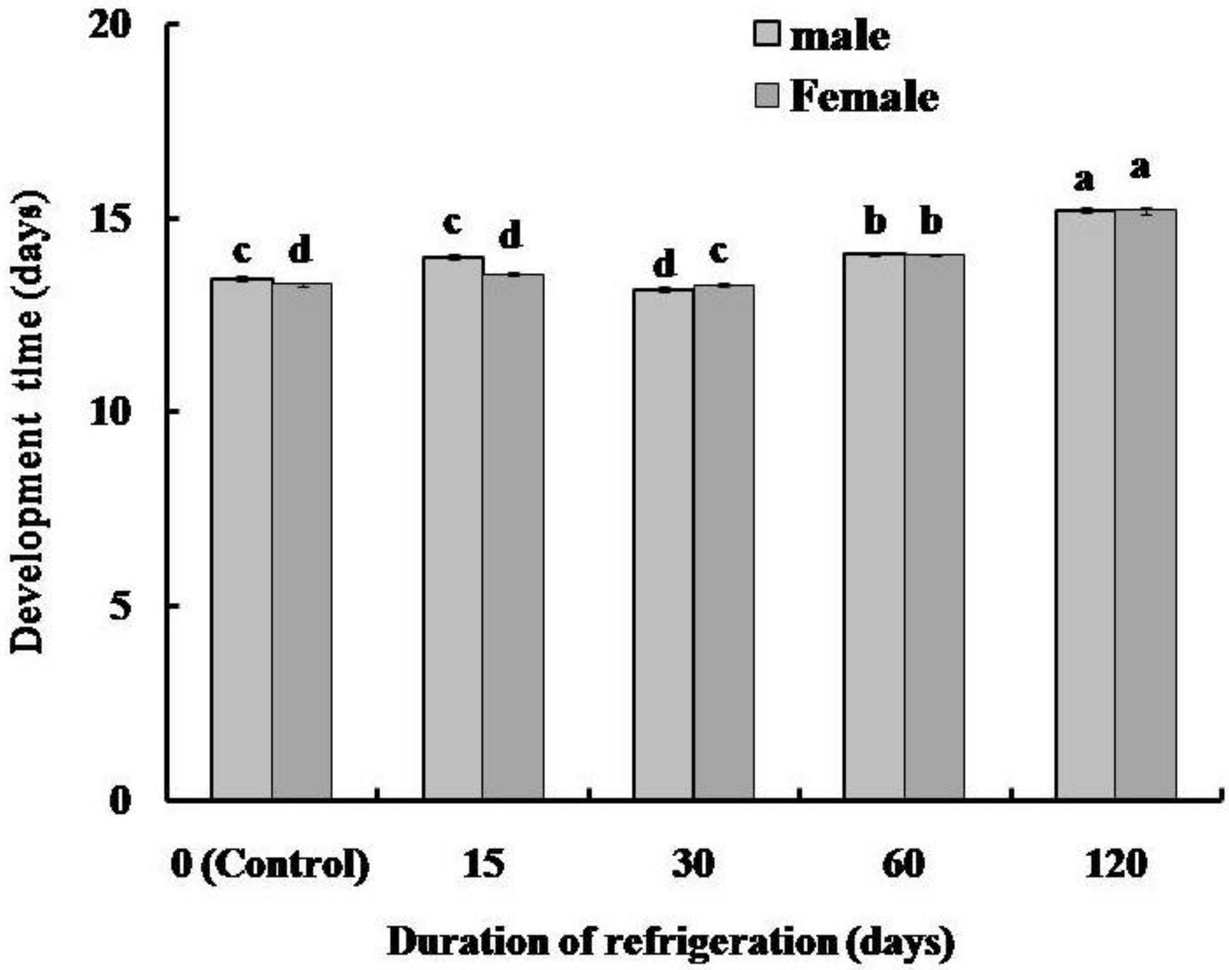

Figure 10. Development time (± SE) of *Ooencyrtus* sp. (male & female) developed in refrigerated egg of *R. pedestris*. Values followed by different letters are significantly different by Kruskal-Wallis single factor analysis of variance by rank ($P < 0.05$).