Journal of Biological Control, 29(2): 75-84, 2015

Research Article

Feasibility of continuous rearing of a potential ichneumonid parasitoid *Campoletis chlorideae* Uchida

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ABSTRACT: The rearing of ichneumonid parasitoids is a great challenge mainly due to the preponderance of male progeny in the continuously lab reared cultures. The issues faced in the continuous rearing of an indigenous ichneumonid parasitoid *Campoletis chlorideae* Uchida (Hymenoptera: Ichneumonidae) were recorded and attempts were made to tackle these challenges by manipulating the rearing processes. Some of the problems could be considerably alleviated by rearing the parasitoids in larger cages measuring 0.52 x 0.52x 0.76 m (with 5 to 8 parasitoid pairs per cage) and by placing these cages in walk-in chambers set at 26±2°C and 70±2% RH. It is a general observation that the culture deteriorates after some generations in the laboratory, but the need was felt to verify the biological parameters which are affected most due to continuous laboratory rearing. The reproductive rate and intrinsic rate of increase were significantly higher in the field collected parasitoids compared to the continuously lab reared ones indicating that bio-deterioration occurs due to continuous rearing. The study also indicated that it would be ideal to bring in wild culture after five generations in the laboratory and rejuvenation is essential after nine generations. Based on the information generated through basic studies, we aim to provide a simple protocol (including cage design) which can be adopted by insectaries or researchers interested in initiating and maintaining the culture of *C. chlorideae*. Measurable biological parameters are also suggested, which can be used to monitor the quality and scale of production.

KEY WORDS: *Campoletis chlorideae, Spodoptera litura*, rearing protocol

(Article chronicle: Received: 08-04-2015; Revised: 16-04-2015; Accepted: 19-06-2015)

INTRODUCTION

Ichneumonid *Campoletis chlorideae* Uchida is a potential indigenous solitary early larval parasitoid of *Helicoverpa armigera* (Hübner) and *Spodoptera litura* (Fabricius), pre-dominant on crops like chickpea, potato, pearl millet, groundnut, pigeonpea and tobacco in India (Pawar *et al.,* 1989, Romeis and Shanower, 1996, Dhillon and Sharma, 2007). *Campoletis chlorideae* plays an important role in the control of *S. litura* infesting crops like soybean, tobacco in Vietnam and China (Zhou *et al.,* 2010; Dang and Hung, 1999). In India, maximum field parasitism of 80% by this parasitoid has been recorded. However, recent surveys have indicated that field parasitism has decreased drastically over the years, probably due to indiscriminate use of chemical insecticides. Being a solitary endo-larval parasitoid, it is a challenge to mass rear this parasitoid. The major problem encountered in the continuous rearing of this parasitoid is the drastic reduction in female progeny, especially during dry summer months. Several factors were reported to affect the parasitism potential, fecundity, longevity and sex ratio of the parasitoid, *viz.* host species, host age, host abundance, climatic conditions, crop on which the host is reared and age of the male and female parasitoids at the time of mating (Gunasena *et al.,* 1989; Venkatesan *et al.,* 1999; Dhillon and Sharma, 2009, 2011; Kumar *et al.,* 2000; Pandey *et al.,* 2009). However, clear protocols are not available on the cage design, the steps to be followed and the optimum conditions to be provided for continuous maintenance of this ichneumonid parasitoid. Through this paper, the attempts made to alleviate the problem of primarily the male biased sex ratio in *C. chlorideae* through manipulation of rearing procedures and conditions are being presented. The paper also examines the issue of bio-deterioration in continuously laboratory reared populations of *C. chlorideae.*

MATERIALS AND METHODS

Host culture

Laboratory reared *S. litura* culture (National Accession No. NBAII) used as the host material for the experiments was from the live insect repository at ICAR-National Bureau of Agricultural Insect Resources (NBAIR), Bangalore, India. The host larvae were maintained on a semi-synthetic diet developed by Nagarkatti and Satyaprakash, (1974).

Parasitoid culture

The initial culture of *Campoletis chlorideae* was obtained from the parasitized *H. armigera* larvae collected from red gram fields. For the laboratory culture of *C. chlorideae,* 2nd instar larva (3 to 4 day-old) of *S. litura* was used. *Spodoptera litura* was identified as the alternate laboratory host as the major target host *H. armigera* is cannibalistic and more prone to NPV infection. Ten pairs of *C. chlorideae* were released into a cage (1 foot wooden cages - 1ft x 1 ft x 1ft with cloth walls on three sides and an acrylic door and with a cloth sleeves attached to one of the side walls). The cages were provided with 50% honey and water - soaked cotton swabs. The mated female parasitoids were utilized for parasitisation after a pre-oviposition period of 24 hours. *Spodoptera litura* larvae were released on castor leaf bouquets and these bouquets with the larvae were exposed to the parasitoids (@ 20 larvae for each female). After 24 hours, the larvae were removed from the bouquets and transferred individually into glass vials (3.5 x 3.0 cm), containing semi-synthetic diet (Nagarkatti and Satyaprakash, 1974). Again, fresh bouquets with the same number of host larvae were placed in the cage.

The parasitized larva in the vial fed on the diet and moved to the wall of the tube or the cotton plug, where the parasitoid maggot moved out of the parasitized larva and formed the cocoon. This generally happened in 10-12 days from the date of parasitisation. The cocoons are dirty white in colour and are either plain or have small black spots on them. The cocoons were later placed separately in one foot wooden cages for adult emergence. When adults emerged, they were sexed and used for continuation of the culture.

Comparison between different parasitoid densities per cage

The production details were evaluated with 3 parasitoid densities per cage *viz*., 5-8, 10-13 and 20-23 pairs per cage. *Spodoptera litura* (3 to 4 days old) larvae were exposed to the parasitoids @ 20 larvae per female for 24 hours. After each exposure, the larvae were transferred from the castor leaves to semi-synthetic diet in glass vials. Fresh set of larvae were then exposed to the parasitoids. The exposures were limited to five and five replications were maintained. Data was recorded on the number of cocoons formed and number of females emerging out of these cocoons. For the three different treatments, data was converted on cocoons harvested from one cage, cocoons produced by each female, number of females emerging in one cage and number of females produced by one female. The data was subjected to one way Analysis of Variance.

Rearing of *Campoletis chlorideae* in small 1 foot cages *vs.* large cages

Production details for *C. chlorideae* were worked out by utilizing two kinds of cages – one foot wooden cages (as described above) and large wooden cages (measuring 1.7 x 1.7 x 2.5ft with cloth walls on three sides and a transparent acrylic pane door and acrylic roof with a cloth sleeve attached to one side of the cage) and compared. Ten pairs of parasitoids were released per cage and provided with cotton swabs soaked in 10 per cent honey solution (3 drops of Vitamin E was added to 50 ml of 10% honey solution). Water swabs were also provided. The adults were allowed to mate for one to two days before allowing them to parasitise 3 to 4 day old *S. litura* larvae which were released on castor leaf bouquets @ 20 larvae for each female parasitoid. Pollen streaks were provided on the inner side of the roof of the cage. Wet sponge pieces in petri plates were kept inside these cages for maintaining humidity. After 24 hours, the castor leaf bouquets were removed from the cages, the larvae were transferred into glass vials containing a semi-synthetic diet. The exposures and collection of cocoons were done as described above. This experiment was conducted between August to March (for two years) and production details were continuously recorded from 10 numbers of one foot cages and ten large cages. Observations were recorded on per cent parasitism (number of cocoons formed out of the total number of larvae exposed), number of cocoons produced per female, per cent adult emergence and number of female progeny produced per female. Host larvae were exposed to the female parasitoids every day and the results are based on the parasitisation, cocoon formation and female progeny from the first five days of parasitisation. This experiment was done to understand the general trend of production if two different kinds of cages are used for rearing *C. chlorideae* and the data was not subjected to statistical analysis.

Rearing of *Campoletis chlorideae* in normal laboratory conditions *vs* rearing in walk-in-chambers

Based on the observation that there is a reduction in female progeny generally during the period January to March, attempts were made to compare the performance details when the parasitoid was reared in large cages placed

in the normal laboratory conditions *vs*. in walk-in-chambers set at 25±2°C and 70±2% RH. Ten pairs of parasitoids were placed in each cage and *S. litura* (3 to 4 days old) larvae were exposed to the parasitoids @ 20 larvae per female. Six replications were maintained for each treatment. The exposures were limited to five and data was recorded on per cent parasitism, number of cocoons harvested per cage and per cent females among progeny. The data was subjected to Analysis of Variance.

Bio-deterioration studies on *Campoletis chlorideae*

a) Fertility parameters of field collected and lab reared parasitoids

Parasitised larvae or cocoons of *C. chlorideae* were collected from chickpea and pigeonpea fields. When adult parasitoids emerged, they were used for parasitizing lab reared *S. litura* larvae. This was recorded as the field collected batch (FC). The adults which emerged from the lab reared cocoons (from the culture which was reared continuously in the laboratory for at least 5 generations) were considered as lab reared batch (LR). In each batch (FC and LR), the adults were allowed to mate and after a preoviposition period of 24 hours, 10 sets of two pairs each were kept in cages with honey and water. Bouquets of castor leaves were placed in the cages with *S. litura* larvae @ 20 larvae (3-4 day old) for each female. The exposed larvae were removed after every 24hr period and a set of fresh larvae were exposed to the parasitoids. The exposures were continued till the mortality of the females and the longevity was noted. The cocoons formed were kept separately and adults emerging from each exposure were counted and sexed. Thus, the fecundity (in terms of progeny produced) was recorded. The experiment was conducted at 26±2°C and RH of 65±2%. The fertility table statistics was calculated using the methods and terminology of Andrewartha and Birch (1954) and were based on the production of female progeny only.

The age specific survival (l_x) and age specific fecundity (m_x) at each pivotal age x were worked out for entire reproductive period. l_x is the number of individuals alive at age x as the fraction of 1, m_x is the number of female offspring produced per female at age interval x. Utilizing these, the following fertility table parameters were calculated.

Net reproductive rate (R_o) = $\Sigma l_x m_x$

Approximate duration of a generation (T_c) = $\frac{\Sigma x l_x m}{R_o}$

Approximate intrinsic rate of increase (r_c) = $\frac{\log_e R_o}{T_c}$

Precise intrinsic rate of increase (r_m) = $e^{-r_m} x l_x m_x = 1$

Net generation time (T) = $\frac{\log_e R_o}{r_m}$

Finite rate of increase (λ) = $anti \log_e r_m$

Weekly multiplication of the population (r_w) = $(e^{r_m})^7$

Hypothetical F_2 females = $(R_o)^2$

b) Parasitism, cocoon formation, adult emergence and sex ratio in field collected and lab reared parasitoids

Helicoverpa armigera larvae (first to third instar) were collected from pigeonpea plots (in the month of September 2012) and were reared in the laboratory on semi synthetic diet. Approximately 20% of the field collected larvae were parasitized and we could obtain cocoons of *C. chlorideae.* These were collected and kept separately for emergence. The batch of adults which emerged from these cocoons was considered as field collected batch (FC). This experiment was conducted to find out the problems, if any, associated with continuous laboratory rearing of *C. chlorideae*. The number of parasitoids in the cage and the method of exposure and number of larvae exposed were as in the previous experiments. Data was recorded on per cent parasitism (based on the number of cocoons formed out of the total number of larvae exposed), per cent adult emergence (based on the number of adults emerged out of the total number of cocoons formed), number of cocoons produced by each female and per cent female progeny amongst the total number of progeny for the field collected adults and also for the adults continuously reared in the laboratory from 6th to the 10th generations (LR6 to LR10). Ten cages were maintained for the FC and for each lab generation. This experiment was conducted to record the comparative trend of performance of field collected and lab reared parasitoids and hence not subjected to statistical analysis.

RESULTS AND DISCUSSION

Comparison between different parasitoid densities per cage

The production details were evaluated with 3 parasitoid densities per cage *viz.* 5-8, 10-13 and 20-23. The mean number of cocoons which could be obtained per cage was 70.4, 69.2 and 100.2, respectively, and mean number of females that could be collected per cage was 14.6, 21 and 34.8, respectively. Though the total number of cocoons and females emerging from each cage was highest in the cages with 20 to 23 female parasitoids released, the mean cocoon production per female and female progeny production per female were highest at 5-8 females per cage, the values be-

ing 14.1 and 5.8, respectively (Table 1).

Table 1. Cocoon and female progeny production at different parasitoid densities per cage

No. of female parasitoids per cage	Cocoon harvested per cage	Cocoons/ female	No. of females per cage	No. of females/ female
5-8	70.4±1.8	14.1±0.3	14.6±1.3	5.8±0.6
10-13	69.2±2.5	5.6±0.4	21.0±0.7	2.0±0.3
20-23	100.2±5.6	6.2±0.2	34.8±1.6	2.4±0.2
Df	2,12	2,12	2,12	2,12
F	22.9	196.79	68.03	26.16
P	<0.0001	<0.0001	<0.0001	<0.0001
LSDat $P \leq 0.01$	15.9	1.5	5.4	1.8

Rearing of *Campoletis chlorideae* in small cages *vs.* large cages

The different biological parameters recorded when *C. chlorideae* was reared in two different types of cages *viz.* small one foot cages and large cages (Fig. 1) during the different months of *C. chlorideae* rearing are as indicated in Fig. 2A to 2D. The number of cocoons produced by each female ranged from 2 to 8.7 when reared in the one foot cages compared to 6.1 to 17.8 in the large cages. A reduction in cocoon production occurred from December in the former, while in the latter it occurred from February (Fig. 2A).

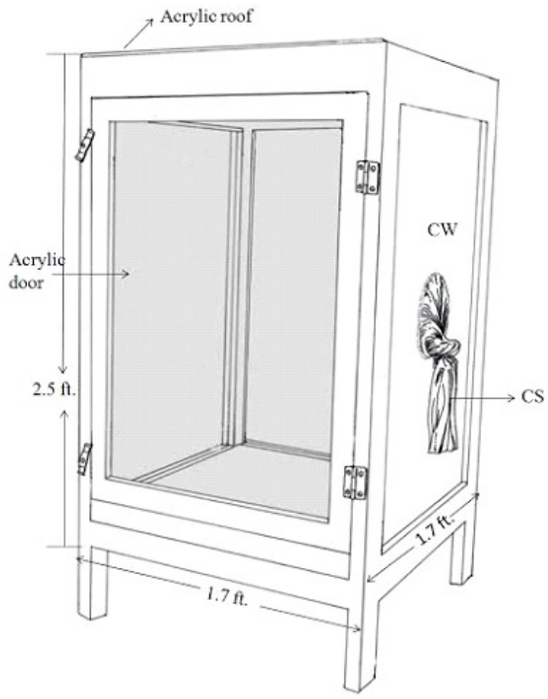

Fig. 1. Large cages used for rearing *Campoletis chlorideae.*

Per cent parasitism ranged from 10.32 to 35.2 in the small one foot cages and 23.5 to 42.4 in the large cages. In the small cages, there was a clear reduction in parasitism from December onwards (Fig. 2B), while in the large cages a per cent parasitism of more than 30% was recorded till March. Per cent adult emergence ranged from 34.8 to

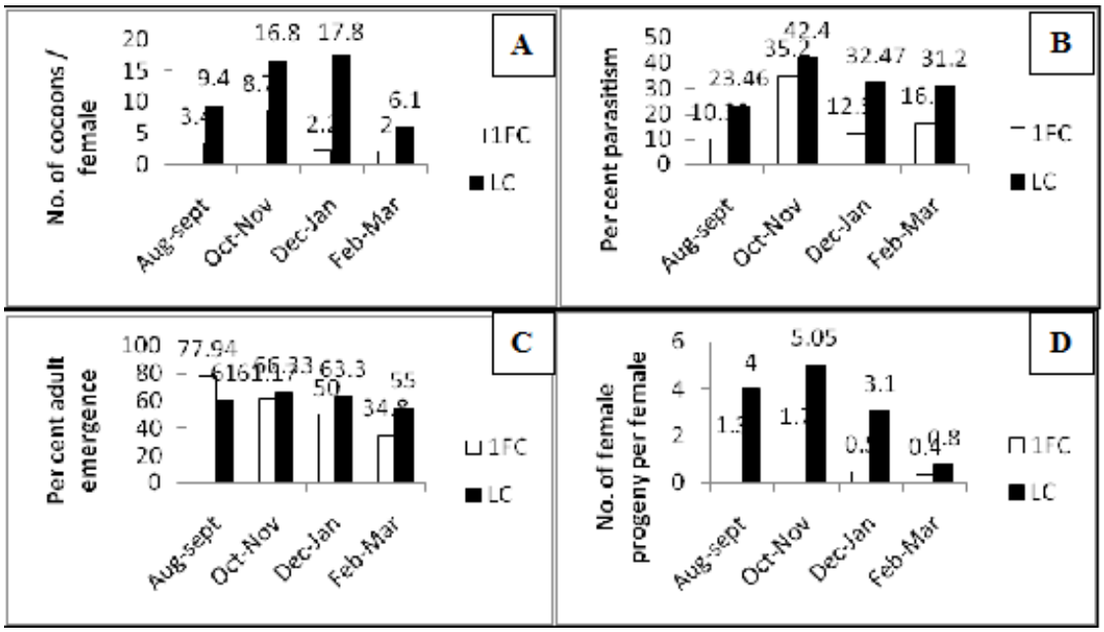

Fig. 2. Comparison between rearing *Campoletis chlorideae* in small one foot cages (1FC) vs. Large cages (LC) with respect to A) No. of cocoons produced by one female B) Percent parasitism, C) Percent adult emergence D) Number of female progeny per female.

77.9 in one foot cages, whereas 55 to 66% emergence was recorded in large cages.

The number female progeny produced per female ranged from 0.4 to 1.7 when the parasitoids were reared in one foot cages, whereas when reared in large cages, 0.8 to 5.05 female progeny per female could be recorded.

Rearing of *Campoletis chlorideae* in laboratory conditions *vs.* in walk-in chambers during dry months

The production parameters, with respect to per cent parasitism and number of cocoons and adult parasitoids that could be harvested from each cage, were not significantly different when the rearing cages were placed in the normal laboratory conditions when compared with those when the cages were placed in Walk-in-chambers set at constant temperature (26±2°C) and humidity (70±5%RH) conditions. However, significantly more number of females (15.3) could be recorded from the latter, compared to the former (5.8) (Table 2).

Bio-deterioration due to continuous rearing

A) Fertility table parameters for lab reared and field collected C. chlorideae

Figures 3A and 3B give the age specific survival and fecundity of field collected (FC) and lab reared (LR) batches of *C. chlorideae*. In both FC and LR, the first mortality occurred on the 6th day of oviposition period and they lived for 11 and 10 days, respectively. In FC, progeny production was higher (3.0 to 4.7 females) during the second to sixth day of oviposition. In LR, progeny production was observed only for the first five days, of which it was more during the first 2-3 days (1.2-1.8). But the females in FC had the capacity to produce female progeny till mortality.

Clear differences were observed between the FC and LR batches of *C. chlorideae* with respect to some of the fertility table parameters (Table 3). The approximate duration of a generation and the net generation time were comparable, 22.21 and 20.64; and 22.01 and 20.11 in FC and LR, respectively. The net reproductive rate was higher (24.4) in FC in comparison to LR (5.1). The approximate intrinsic rate of increase and precise intrinsic rate of increase were 0.14 and 0.15, respectively in the case of FC, whereas in LR the corresponding values were 0.08 for both parameters. The finite rate of increase was 1.16 in FC and 1.08 in LR. Based on these parameters, the weekly multiplication rate was calculated which was 2.76 and 1.76, respectively in FC and LR. The FC batch could double its population in 4.66 days, whereas in LR batch the doubling time was 9.01 days. The hypothetical F2 females were higher in FC (595.36) in comparison to LR (26).

B) Biological parameters of field collected and lab reared C. chlorideae

The performance parameters of field collected parasitoids and those which were reared for more than five generations in the laboratory is indicated in Fig. 3. Mean per cent parasitism was 31.28% in the field collected *C. chlorideae*, whereas the parasitism ranged between 16.7 to 26.14% in the parasitoids reared in the laboratory for six to ten generations. One field collected female produced 61.7 cocoons, whereas a lab reared female produced 14 to 32 cocoons. Continuous laboratory rearing did not seem to affect the per cent adult emergence from the cocoons, which was 58.87% in the case of field collected adults and ranged from 44.79 to 67.86% in lab reared parasitoids. The parameter which was drastically affected by continuous lab rearing was the per cent females among the progeny produced (Fig. 3). Among the progeny produced by the field collected adults, 77.2% were females, whereas in the case of laboratory reared parasitoids the per cent females among the progeny produced ranged between 12.5 to 21.4, indicating a clear male biased sex ratio.

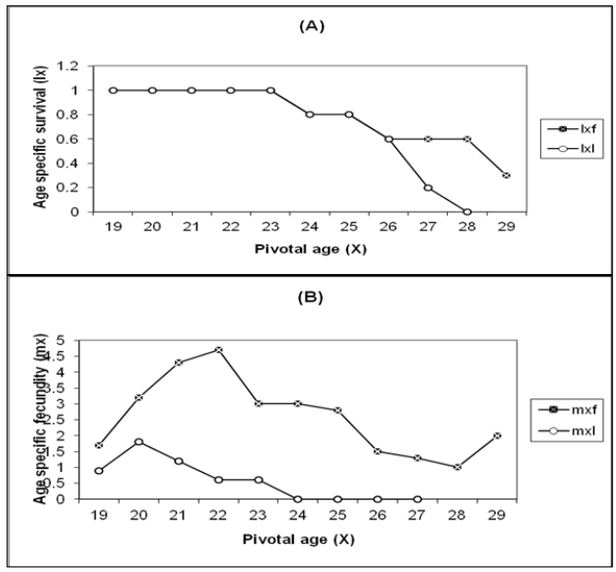

Fig. 3. Comparison between field collected and lab reared *Campoletis chlorideae* with respect to (A) Age specific survival and (B) Age specific fecundity l_xf and l_xl: Age specific survival of field collected and lab reared, respectively m_xf and m_xl: Age specific fecundity of field collected and lab reared, respectively.

It is important to develop a protocol for continuous rearing of *C. chlorideae* as it is a potential parasitoid of both *H. armigera* and *S. litura* in India, Japan, China and Vietnam (Zheng and Lu, 1981; Dai, 1990; Nandihalli and Lee, 1995; Kumar *et al.,* 2000; Liu *et al.,* 2004). The current study was aimed at increasing production efficiency

Table 3. Comparison of the fertility table parameters for lab reared and field collected *Campoletis chlorideae*

Campoletis chlorideae population	R_o	T_c	r_c	r_m	T		Weekly Multiplication rate	Doubling -time (days)	Hypothetical F_2 females
FC	24.4	22.21	0.14	0.15	22.01	1.16	2.76	4.66	595.36
LR	5.1	20.64	0.08	0.08	20.11	1.08	1.76	9.01	26.00

in rearing of *C. chlorideae* by improving parasitism and female offspring production, with minimum labour and cost. An alternate easy to rear laboratory host *S. litura* was chosen for rearing *C. chlorideae.* Though *H. armigera* is known to be a preferred host of *C. chlorideae,* rearing on *H. armigera* is onerous as it is cannibalistic and more prone to viral infection. Through the study it was concluded that for optimum utilization of the parasitoids and host insects, 5 to 8 pairs of *C. chlorideae* could be released into each large cage (Fig. 1) and around 150 *S. litura* larvae (3 to 4 day old) can be released on bouquets of castor leaves and placed inside the cage and allowed for parasitisation for 24 hours. Five such exposures can be made for each cage with parasitoids. Our fertility parameter studies have clearly indicated that female progeny production were restricted to the first five days of the oviposition period though the parasitoid could live up to 10 days. Similar observations were made on *Campoletis sonorensis* (Cameron), with effective oviposition period as 18 days, while the parasitoid lived for 23 days (Murillo *et al.,* 2012). We could record a maximum parasitism, cocoon production/female and female progeny production/female as 42.4%, 17.8 and 5.05, respectively. Gupta *et al.,* (2004) recorded superior biological parameters of *C. chlorideae* when reared on *H. armigera.* However, it appears that the biological parameters were evaluated on the progeny of field collected population of *C. chlorideae.* This is in corroboration with our observations on superior biological parameters in the field collected population of *C. chlorideae* (Fig. 4). The optimum parasitoid: host ratio suggested for *C. chlorideae* with *H. armigera* larvae as host was 1:40 by Nikam (1981) and Gupta *et al.,* (2004). We suggest a ratio of 1: 20 to 30, when *S. litura* larvae are used as host.

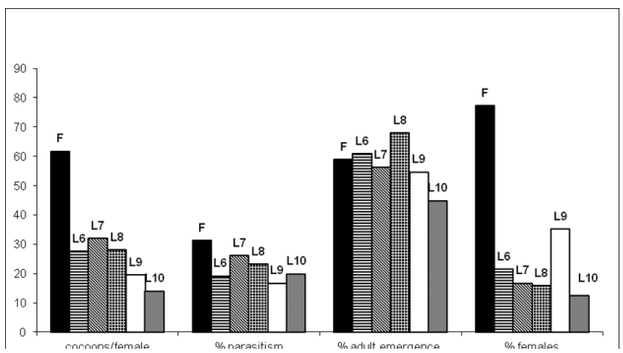

Fig. 4. Biological parameters of field collected and lab reared *Campoletis chlorideae* (F: field population; L6 – L10: sixth to 10th laboratory generations).

Research reports indicate different methods adopted for rearing ichneumonid parasitoids *viz.* single parasitisation (Dhillon and Sharma, 2011), double parasitisation (Zhang *et al.,* 2010) or moderate super parasitisation (Gonzalez *et al.,* 2007). In the current study, we allowed the host larvae to feed on castor leaves and then exposed them to the parasitoids, thus allowing the mated parasitoids to choose the larva for parasitism. Earlier studies have also indicated that larvae feeding on the host plant were more attractive to the parasitoid than freshly released host larvae, probably due to the chemical cues released by the damaged host plants and the larval faecal matter (Bajpai *et al.,* 2002).

Johns and Whitehouse (2004) reared ichneumonid parasitoid *Heteropelma scaposum* (Morley) in different kinds of cages (small, medium and large), but the production parameters were not compared. The current studies indicated that large cages (Fig. 1) are ideal for rearing *C. chlorideae.* Probably they allowed the adults to search for the ideal mating partner. Moreover, bouquets of castor leaves (which are easily available in wastelands in our region) in the adult cages provided the wasps with resting sites and prevented the wasps from aggregating in the corners of the cages. Besides, the damaged leaves and the larval faecal matter attracted the adult parasitoids towards the larvae for parasitisation (Ballal *et al.,* 1996; Bajpai *et al.,* 2002; Murugan *et al.,* 2000).

Methods used to mass rear could often negatively affect the quality of the insects, especially leading to detrimental sex ratio changes. Several factors are known to influence the progeny sex ratio *viz.* climatic condition, host insect quality, density of the host insect and parasitoid, host crop, host age, parasitoid age at the time of mating, parent sex ratio, super parasitism, rearing temperature, etc. (Gunasena *et al.,* 1989; Godfray, 1994; Venkatesan *et al.,* 1999; Dhillon and Sharma, 2007, 2009, 2011; Kumar *et al.,* 2000; Pandey *et al.,* 2009; Venkatesan *et al.,* 1999). However, when insect cultures are to be maintained continuously in insectaries, it would be cumbersome and labour-intensive to precisely choose particular host age or parasitoid ages, host density, parasitoid density, etc., especially if the culture maintenance is done by non-entomologists. Simple protocols and procedures are essential which can be followed for continuous maintenance during different seasons when there are fluctuations in temperature and hu-

midity conditions. We have tried to evaluate the existing methods and attempted to modify and improve them for continuous rearing of *C. chlorideae* in an insectary managed by non-entomologists.

Reports say that when parasitoids are reared on inferior hosts, there is a tendency for production of male rather than female offspring (Charnov *et al*., 1981; Godfray 1994; Quicke 1997). Liu *et al.,* (2004) reported that cocoon production was 55.3% when reared on *S. litura* and 90% on *H. armigera.* We chose *S. litura* as it is a convenient laboratory host, however, a question which arose was whether the biased sex ratio could be a result of rearing on an inferior host. We have recorded parasitized *S. litura* larvae from tobacco fields, which have yielded large number of female *C. chlorideae* (Chandish R Ballal, unpublished). Hence, it would not be appropriate to consider *S. litura* as an inferior host.

Our preliminary studies indicated that single parasitism on *S. litura* larvae was inferior (with respect to female progeny production) to allowing female parasitoids to search and parasitise *S. litura* larvae released on castor leaves for 24 hours (Ballal *et al.,* 1996). Through our studies, we identified the optimum parasitoid: host ratio for mass rearing with a specific exposure time. Probably moderate superparasitism may be advantageous with respect to progeny production and female sex ratio. This is in agreement with the observations of Gonzalez *et al.,* (2007). Hegazi and Khafagi (2008) and Zhang *et al.* (2010) reported that the optimal way for solitary parasitoids to deal with low quality hosts might be to super-parasitise them and this was thought to provide a better host environment for the development of the parasitoid. However, Zhang *et al.,* (2014) reported that super-parasitism does not affect female sex ratio based on his studies on the braconid wasp Meteorus pulchricornis (Wesmael). Whether superparasitism in *C. chlorideae* would precisely help in improving sex ratio would be an aspect to be investigated in depth.

It was observed that the percent humidity recorded was 40 or less from December to next March and during this period, there was a marked reduction in female progeny production, leading to culture deterioration. This could be because of the low humidity conditions which is known to hamper mating. The production of *C. chlorideae* could be continued even during these unfavourable months by rearing in walk-in chambers set at 26±1°C and 70±5% RH.

Ichneumonid parasitoids are generally supplied in the cocoon stage and considering the issue of male pre-ponderance in laboratory reared *C. chlorideae,* it is important to ensure that sufficient number of female cocoons are supplied. Male and female cocoons of *C. chlorideae* cannot be segregated based on morphological characters. Ballal *et al.,* (2000) suggested that if an index *viz.* length x weight of a cocoon in mm and mg, respectively, is 50 or more, 50% of such cocoons can be expected to yield females.

Male biased sex ratios are commonly observed in inbred populations of several ichneumonids and braconids (Cook 1993a). Single-locus complementary sex determination (SL-CSD) was reported to be one of reasons for this, however, according to Stouthamer *et al.,* (1992) SL-CSD does not occur in all ichneumonids. This is an aspect to be investigated in the case of *C. chlorideae* though Dhillon and Sharma (2011) stated that there is a genetic regulation of sex ratio in *C. chlorideae*. Schneider and Vinuela (2007) reported that the male bias if related to the Sl-CSD model, could be mitigated in the ichneumonid *Hyposopter didymator* (Morley) by adopting a laboratory rearing strategy, which uses a single large population and several inbred lines as reservoirs of alleles. We followed a rearing procedure for *C. chlorideae* by randomly mixing the adults emerging from several inbred lines. However, a systematic study would be required to understand if precisely following a laboratory rearing strategy as adopted by Cook (1993b) and Schneider and Vinuela (2007) would improve the sex ratio in *C. chlorideae.*

During continuous laboratory rearing of *C. chlorideae,* deterioration of biological and fertility table parameters could be observed in comparison to the field collected population. This was true especially with reference to female progeny production beyond five continuous generations in the laboratory. Hence we could infer that after every five generations in the lab, rejuvenation with wild population would be important. According to Stouthamer *et al.,* (1992), the chance of obtaining homozygotic males increases when a small sample of individuals is used to initiate a culture and the fewer generations they are maintained in the lab, the fewer alleles are lost. In continuous rearing of *C. chlorideae,* what needs to be investigated in depth in insectaries located in different countries is the optimum frequency of rejuvenation with wild culture to be followed through measurement of the performance of the rejuvenated culture at regular intervals. Deterioration of other ichneumonid cultures with respect to parasitism or female progeny production has been reported by (Schneider and Vinuela, 2007; John and Whitehouse, 2004; Rapport and Page, 1985). In the case of *Hyposoter didymator,* through infusion of wild stock at regular intervals, female progeny production could be improved (Schneider and Vinuela, 2007), while John and Whitehouse (2004) reported

that adding parasitised wild-caught caterpillars to laboratory colony of the *Heteropelma scaposum* (Morley) could not improve the sex ratio. These results indicate that some other mechanism may be causing the sex ratio bias or that the genetic diversity of the additional wild population was not sufficient to offset the production of diploid males. Hence the greatest challenge would be to identify the precise mode of rejuvenation of laboratory culture of *C. chlorideae*.

Realised fecundity and fertility are important factors to be considered not only for initiating a culture, but also for continuing mass rearing systems. Our study focuses on measuring the performance parameters of the field population and continuous lab reared *C. chlorideae*. A parasitoid is considered to be efficient if the potential maximum rate of population increase (r_m) is equal to or larger than that of the host (Van Lenteren and Manzaroli, 1999). If we consider two of the target pests, *S. litura* and *H. armigera,* the r_m of *S. litura* varied between 0.11 (on groundnut *Arachis hypogoea*) and 0.20 on sunflower (*Helianthus annus* L) (Garad *et al.,* 1984), while in the case of *H. armigera,* r_m value of 0.14 was recorded on cotton and chickpea (Acharya *et al.,* 2007; Dabhi and Patel, 2007). In our study, r_m of field collected population of *C. chlorideae* was 0.15 and after five generations in the laboratory, reduced to 0.08. This indicates that the field population of *C. chlorideae* would be effective against the above pests infesting different crops, however laboratory rearing can reduce the reproductive potential of the parasitoid, making it less effective. Pandey and Tripathi (2008) have reported higher r_m values ranging between 0.08 and 0.279 while rearing *C. chlorideae* at different temperature regimes. However, the superior biological or fertility parameters of *C. chlorideae* reported by earlier researchers could be due to the fact that either field collected adults or those from the first laboratory population were used for the experiments and not the continuously reared parasitoids as in the current experiment.

Insectaries can adopt two simple yard sticks to measure the quality of their culture: 1) number of cocoons harvested from each cage holding 5 to 8 pairs of *C. chlorideae* 2) number of females obtained from one cage - based on five parasitisations for each cage. Based on the above record, cocoon production and female progeny production by each female can be calculated. The aim should be to obtain a minimum of 40 cocoons and 15 female progeny (or 40% female progeny) from each female. When the female progeny production goes below 5 by each female, infusion of wild culture should be considered as essential for rejuvenation. In Indian conditions, it would be appropriate to bring in wild *C. chlorideae* culture during November end to avoid detrimental sex ratio changes.

Through this study, a simple protocol has been developed, which can be followed by insectaries interested in continuously rearing *C. chlorideae*. In Indian conditions, the months August to November appear to be conducive for rearing *C. chlorideae* and during this period, the parasitoids could be released in larger numbers against *H. armigera* infesting chickpea and pigeonpea. Focus should be on evaluating augmentative releases with smaller numbers of mated females, timing the releases to precisely target the early instars of *S. litura* and *H. armigera* infesting different crop ecosystems.

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