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Evaluation of quality of *Trichogramma chilonis* Ishii from different production units in India

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ABSTRACT: **The post-shipment quality of parasitized egg cards of *Trichogramma chilonis* Ishii obtained from nine production units in South India was evaluated. Evaluations included the number of parasitized eggs per card, per cent parasitism, per cent adult emergence, sex-ratio and parasitizing efficiency on the target host. There was a lot of variation in the card size and the number of eggs per card. The parasitism was more than 90 per cent in the tricho-cards supplied by two units. The adult emergence and sex-ratio was as per the quality standards. Considering the recommendation of 1,50,000 parasitized eggs per hectare of cotton, only three units could reach the quality standards. The parasitizing efficiency of *T. chilonis* supplied by two units was less than 50 per cent and parasitism ranged between 60 and 73 per cent in the others. There is a need for regulatory agencies to periodically evaluate the quality of Trichogrammatids produced by insectaries based on simple and reliable methods. Trichogramma producers should reach a consensus with respect to the method of producing Tricho-cards and recommendations to be given to farmers. This would ensure the proper utility of Trichogrammatids in biocontrol programmes.**

KEY WORDS: *Helicoverpa armigera*, quality evaluation, *Trichogramma chilonis*

INTRODUCTION

The effectiveness of biological control dependents on the quality of natural enemies produced by mass rearing units. An Infobase prepared by the Project Directorate of Biological Control (PDBC), Bangalore (Biswas *et al.*, 2004) reports that there are 128 units producing bio-agents in India and a maximum of 34 are involved in the production of various species of *Trichogramma*, mainly *T. chilonis* Ishii *T. chilonis* is included as one of the components in the IPM modules for various pests in the cotton, sugarcane and vegetable ecosystems (Singh, 2002). Augmentative releases of trichogrammatids have not always been successful. Hoy *et al.* (1991) reported that releases made with poor quality natural enemies or fewer natural enemies than needed could lead to control failures. Although there are several research papers describing the methods to be followed to assess the quality of mass produced natural enemies (Huetell, 1976; Boller and Chambers, 1977; Hassan *et al.*, 1991; Bigler *et al.*, 1993; Leppla, 1994), there are very few studies, especially in India, which assess the quality of mass produced natural enemies as received by customers (Losey and

Calvin, 1995; Romeis *et al.*, 1998; Hassan and Zhang, 2001).

The present study was taken up with an aim to assess the post-shipment quality of mass produced *T. chilonis*. *T. chilonis* was chosen for the study, as it is mass-produced by several mass production units in India and also widely used in IPM modules. In this paper, we present the results of an assessment of selected aspects of quality (measured in terms of some biological parameters) of *T. chilonis* supplied by some production units in southern India.

MATERIALS AND METHODS

The parasitized egg cards of *T. chilonis* (Tricho-cards) were obtained during 2000 and 2001 from nine sources: four commercial producers, four Government laboratories and one NGO. A set of 3 cards was received from each supplier twice during 2000 and 2001. The first set of cards received was used to assess the quality of the cards and the second for assessing the parasitizing efficiency. The tricho-cards supplied by each unit were evaluated for the following parameters:

Total no. of eggs/ card: Initially the card size was measured. At 15 randomly selected spots on a card, the number of eggs per 0.25 cm^2 was counted and number obtained was then extrapolated to the full size of the card.

Per cent parasitism: While counting the number of eggs as mentioned above, the parasitized and unparasitized eggs were counted separately to calculate the per cent eggs parasitized.

Per cent adult emergence: Five egg card bits, containing 200 parasitized eggs each was selected randomly from the tricho-cards supplied by each unit. These bits were placed individually in test tubes and the tubes were plugged tightly with cotton wool. After adults emerged from the parasitized eggs, the number of eggs with emergence holes was counted to calculate the percent adult emergence.

Sex-ratio: From the adults emerged, the number of males and females were counted to arrive at the sex-ratio.

Parasitizing efficiency on target host: one hundred and twenty five *Helicoverpa armigera* eggs were placed on a bouquet of cotton leaves in a 30 cm cage. Five *T. chilonis* females were released into each (for obtaining a parasitoid-host ratio of 1: 25). After 24 hours, the *H. armigera* eggs were collected and pasted on a card strip using water. After a week, blackened eggs were counted, based on which per cent parasitism was worked out. Two units (B and F) did not supply the cards for this study.

Date of emergence: The difference between the expected date of adult emergence, which was provided by the supplier, and the actual date of emergence was calculated.

Recommendations: The recommendations provided by each company for releases on cotton such as number of cards/ release/ hectare, number of releases and interval between releases and any other information provided were carefully examined.

Rate of release: From the data on the number of parasitized eggs per card, the adult emergence (%) and the number of cards recommended per release per hectare by each company, the actual number of trichogrammatids being released per hectare was calculated for each unit. The number of parasitoids emerging per card was based on the assumption that one parasitoid per host egg emerged, though more than one progeny can develop in one egg of *Corcyra cephalonica* Stainton (Chacko, 1969).

RESULTS AND DISCUSSION

Singh *et al.* (2001) have listed out the quality parameters for trichogrammatids, which include clear species identification, per cent parasitism and per cent emergence $\geq$90, per cent females 50 or more and one card to contain 16000 to 18000 eggs. In the present study, all the production units that supplied the tricho-cards reared *T. chilonis* on UV-irradiated eggs of *C. cephalonica*. It was observed that there

was a wide variation in the size (from 45 to 168 cm^2) of tricho-cards supplied by different producers. The number of eggs per card ranged between 10,257 and 26,921 and Units C, D and G reached the required standard of 16,000 to 18,000 eggs per card. Romeis *et al.* (1998) also observed wide variations in the number of eggs per card when they examined Tricho-cards supplied by different insectaries.

Jalali *et al.* (2003) recommended 1,50,000 parasitised eggs of *T. chilonis*/ hectare /release and 6 to 8 such releases for management of bollworms on cotton during the crop season. If each card holds around 17000 parasitized eggs, 9 such cards are required for one hectare of cotton. Hence, it would be advantageous for all the units to produce tricho-cards of uniform size, which could conveniently hold 18,000 - 20,000 eggs.

It is essential for the farmer to have the information regarding the expected date of emergence of adults from the cards. This would enable him to stape the cards in the field just before adult emergence and thus prevent, to some extent, predation of the parasitized eggs by naturally occurring predators. Units B, F, G and I did not provide the information on the expected date of emergence. The other five units provided the information, but the actual date of emergence was delayed by 1 to 4 days.

It was observed that per cent parasitism was more than 90 in the cards supplied by two units, i.e. B and F (Table 1). This was on par with the per cent parasitism recorded in cards from units 4 and 8 (83.04 and 82.21, respectively). This was followed by units A, G and E. Minimum parasitism of 67.39 per cent was recorded in the cards supplied by unit I.

There was no significant difference between the cards supplied by different units with respect to mean per cent adult emergence (Table 1), which ranged from 60.48 in the case of unit H to 100 in the case of units D, E and G. It was observed that in majority of the cards more than one adult emerged from a single parasitized egg, thus resulting in a high per cent adult emergence in most of the cards. Emergence levels in the field are generally lower than that in the lab (Bigler *et al.*, 1993). Hence it is important to have a higher per cent adult emergence from the parasitized eggs.

In the present study, per cent females emerging from the different tricho-cards ranged from 51 to 62, all being statistically on par. A balanced sex-ratio of the adults emerging from the tricho-cards is included as one of the quality parameters by Singh *et al.* (2001) and the International Organization for Biological Control (IOBC) has set a sex-ratio of $\geq$50 (F: M) as a quality standard for *Trichogramma brassicae* (Lantern *et al.*, 2003). Hassan and Zhang (2001) reported that per cent females in the *T. brassicae* supplied by commercial suppliers in Germany was more than the IOBC standard.

The methodology to be followed for *Trichogramma* releases is an important aspect, which should be provided to the end user along with the Tricho-cards for their proper utilization. This was provided only by units C, D, E and F. The general method to be followed for the *Trichogramma* releases is for the card to be cut into bits and fixed on to the leaves (Singh *et al.*, 1994). Tricho-cards from units C, D, E, H and I had lines and perforations on them which would enable the farmers to cut the cards into tricho-bits and distribute them in the field. Cards from units A, B and G had lines on them, but no perforations, while those from unit F had neither. Variations were observed in the number of bits each card could be divided into, 3 to 16 bits. Unit D was the only unit that supplied tricho-cards, which could be cut into 16 bits (following the recommendations made by the All India Co-ordinated Research Project on Biological Control).

A wide variation was observed in the recommendations provided by different units with respect to the number of cards to be released/ hectare (ranging from 3 to 12), number of releases to be made per hectare (ranging from 3 to 10) and the time interval between the releases (ranging from 6 to 15 days) (Table 1). Only four units (A, D, F and G) recommended 9 cards for cotton crop (which is as per the standard recommendation). Most of the

Table 1. Some measurable quality parameters of *T. chilonis* egg cards supplied by different production units

Production Unit	Card size (cm^2)	Eggs/ card	Parasitization (%)	Adult parasitoid emergence (%)	Variation of the actual DOE from the expected DOE (Delayed by days)	Per cent females	No. of cards recommended/ ha(No. of releases/ha)	Release interval (days)	Parasitizing efficiency on target host
A	96.00	14105.28	80.00	80.87	3	54.47	9 (6-8)	7	64.53
B	60.00	13584.00	92.81	77.58	NP	56.76	3-5 (10)	10	(CNS)
C	132.00	18057.60	77.34	95.29	2	55.87	5-12 (#)	10-15	63.25
D	73.50	17836.25	83.04	100.00	1	57.36	9 (6)	15	73.47
E	57.12	13464.90	79.00	100.00	4	51.72	3-5 (3-4)	10-15	66.00
F	45.00	10256.85	91.23	91.78	NP	62.38	9 (3)	6	(CNS)
G	101.36	26921.22	79.51	100.00	NP	57.93	9 (6-8)	7	60.13
H	168.30	15333.25	82.21	60.48	2	54.09	5-12 (NP)	NP	37.13
I	75.00	12679.00	67.39	77.26	NP	51.26	5-12 (NP)	NP	46.72
CD (P<0.05)	*	9092.35	10.92	NS	*	NS	*	*	8.24

* : Not subjected to statistical analysis

\# : Throughout the egg laying period

(CNS) : Cards not supplied by the company for the study on parasitising efficiency

NP : Information not provided by the producer

DOE : Date of Emergence

units did not provide the information on how many cards have to be released for specific crop ecosystems. Two of the units did not provide information on the number of releases to be made and the time interval between releases. Unit C recommended that releases have to be made throughout the egg laying period of the pest. It would be difficult for a farmer to monitor the egg laying period of the pest and to follow this recommendation.

In any successful biological control release program, it is necessary to ensure that a specific number of parasitoids are released per unit area. Considering the AICRP recommendation of 1,50,000 parasitized eggs per hectare (Singh *et al.*, 1994), units C, G and H could reach this standard (Fig. 1) (in the case of G and H, when 12 cards were used per/ ha). Though the per cent parasitism in these cards was not more than 90, more number of cards are recommended per hectare by these units, resulting in the requisite number of parasitized eggs to be released.

The optimum number of females to be released per hectare is 75000 (considering the release rate of 1,50,000 parasitised eggs/ ha and 50% female progeny). Only units C, D and G could reach this standard. In the case of the other units, the number of parasitized eggs actually released was 11 to 65 per cent less than the AICRP recommendation. Though source H could reach the standard with respect to number of parasitised eggs released per hectare, because of the low per cent adult emergence (60.48), the number of females released was only 50,000 (Fig. 1). Based on their evaluation of tricho-cards supplied by different units in South India, Romeis *et al.* (1998) observed that due to the low product quality, up to 85 per cent fewer parasitoids were being actually released than claimed by the producer.

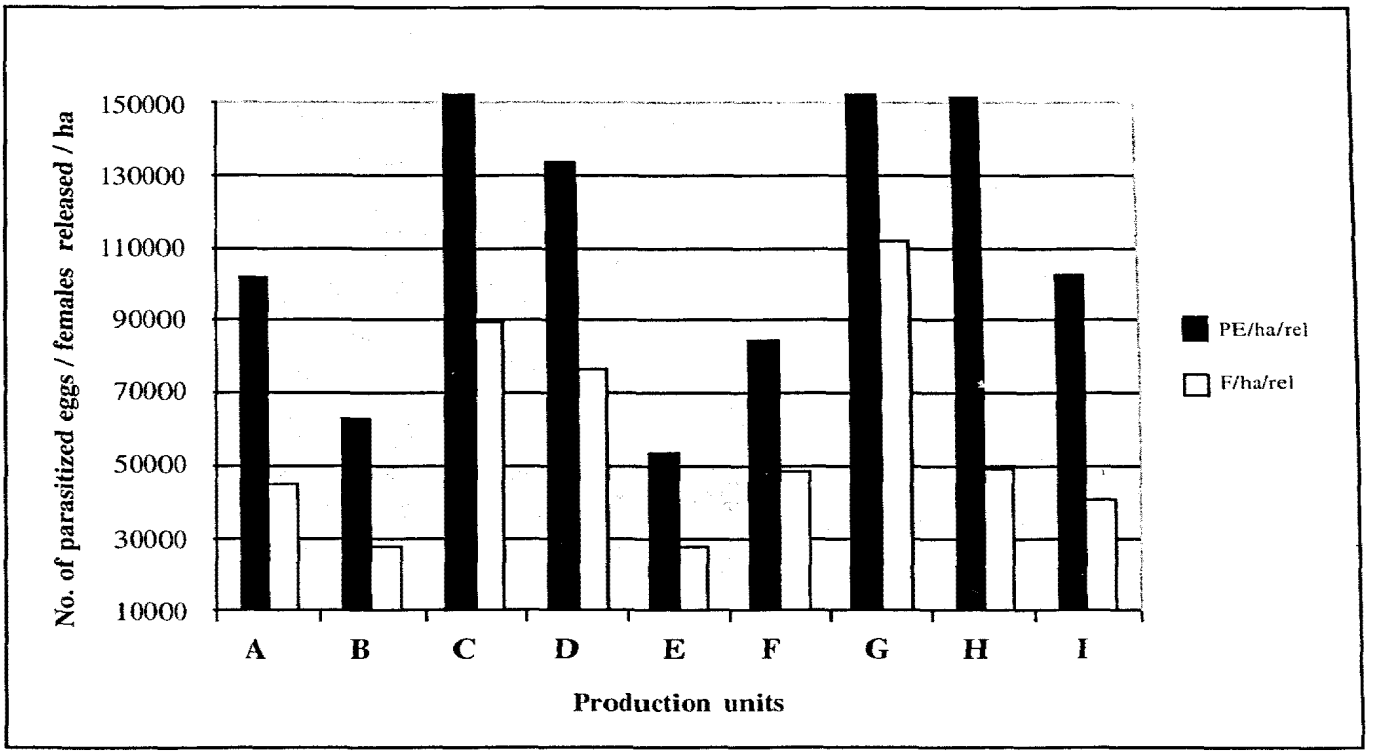

Figure 1. Number of parasitized eggs (PE) released/ ha/ release (rel) and number of females released/ ha/ release

Trichogrammatids reared on alternate laboratory hosts may fail to search and parasitize the target host efficiently. In the present study, the ability of the trichogramma adults (obtained from different units), reared on alternate laboratory host, *C. cephalonica*, to search and parasitize the target host (*H. armigera*) is presented in Table 1. The parasitizing efficiency was highest in the case of trichogrammatids obtained from units D and E (73.47 and 66%, respectively). This was followed by the parasitism by those from unit A (64.53%), which was on par with those from units C and G (63.25 and 60.13%, respectively). The trichogrammatids emerging from the tricho-cards supplied by units H and I were significantly inferior to those supplied by other sources with respect to per cent parasitism (37.13 and 46.72, respectively) (Table 1). The reduction in the parasitizing efficiency could have been due to continuous rearing on the laboratory host, *C. cephalonica* or supply of cards which were stored for longer durations. Singh *et al.* (2001) have suggested that the tricho-cards should not be stored for more than 2 weeks at 10° C and *C. cephalonica* eggs for not more than 5 days at 5° C. The reduction in preference for natural host when reared continuously on laboratory host has been reported for *Trichogramma evanescens* Westwood (Hassan *et al.*, 1991) and for *Trichogramma brassicae* (Bergeijk *et al.*,1989).

Based on the present study, the following have been suggested

- It is desirable that all *Trichogramma* production units in the country form a working group with the help of research organizations and university departments to finalize the standards for preparation of Tricho-cards and to formulate the recommendation for field release. They should follow this uniform standard while producing cards with respect to card size and design, number of eggs per card, per cent parasitism, per cent adult emergence and sex-ratio.
- The quality parameters: per cent female progeny ≥50, per cent parasitism and per cent adult emergence ≥90 to be followed strictly by all companies.
- When Tricho-cards are supplied, the expected date of emergence is to be mentioned to enable the end user to release the card in the field just prior to adult emergence.
- Though the stipulated standard is for each card to contain 16000 to 18000 parasitized eggs, the ideal situation would be for each card to contain 18000 to 20000 eggs per card to make up for any reduction in parasitism, adult emergence and female progeny. To ensure the release of adequate number of parasitoids per hectare, the supplier can also provide an extra card for every nine cards required for one hectare.
- Uniform standard crop-based recommendations, with respect to number of cards per hectare, number of releases per hectare, time interval between releases, the number of bits to be cut from each card, and method of fixing the cards in the field to be provided by all producers while supplying Tricho-cards.

The present study clearly indicates that the quality of tricho-cards supplied by different units is highly variable. The major problem was that the number of parasitized eggs that was being released per hectare was much lower than the actual recommendation. The need of the hour is to formulate a set of standard laboratory methods, which should be used by all production units to detect deterioration of the trichogrammatid cultures and initiate corrections on time. A set of standard recommendations has to be formulated, which should be provided by all production units in the country when they supply their Tricho-cards.

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