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Preliminary testing of some new release methods for egg parasitoid *Trichogramma* spp.

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ABSTRACT: **Preliminary investigations were conducted in laboratory cages to test the efficiency of a novel release technique for egg parasitoid *Trichogramma* species and this was compared with the traditional techniques. The efficiency of the techniques was determined based on time taken for release, adult emergence and percentage egg parasitism. Mixing of eggs with carriers like talc (1:1 ratio) and agar solution (0.1%) recorded least time for application. Spraying of loose parasitised eggs mixed with agar solution was considered as the best treatment amongst the treatments where aqueous solutions were used as carriers. In treatments where loose parasitised eggs were mixed with various solid and aqueous carriers, per cent emergence was low ranging from 40.5 to 57.1% compared to 95.0 to 100.0% in release of adults, *Tricho* bit, *Tricho* capsules and loose eggs without any carrier. The lowest emergence was recorded when loose parasitised eggs were mixed with talc. Adult release, sprinkling of loose parasitised eggs mixed with vermiculite and semolina gave significantly higher parasitism compared to the other techniques of release. The results indicated that release of *Trichogramma* can also be tried by mixing with a spray solution (for eg. agar solution) with modification of sprayer nozzle or with solid carriers (for eg. vermiculite). Such techniques will be better understood by stakeholders and is expected to increase uptake of this important biological control agent.**

KEY WORDS: Carriers, emergence, parasitism, release method, *Trichogramma*

INTRODUCTION

The effectiveness of pest control by *Trichogramma* is determined by many factors such as species of the *Trichogramma* used, quality of the parasitoid product, number of releases and timing of releases, release techniques and complex interactions between the parasitoid, the target pest, the crop and the environmental conditions (Knutson, 1998). Among these factors, techniques of release can influence the behaviour of *Trichogramma* and can affect the level of pest control achieved (Keller *et al.*, 1985). *Trichogramma* can be released in many ways, either from ground or air, point distribution or broadcast form (bioresources.com – personal communication). However, in India *Trichogramma* are generally released as parasitised eggs in the form of *Tricho*

bits or as adults. There has been no attempt to explore the possibility of releasing *Trichogramma* through other techniques. Since the prime stakeholders of biological control agents are farmers, release method such as dry formulation or as spray solution will be better understood than the present traditional techniques advocated. Keeping this in mind, a preliminary attempt was made to test the effectiveness of novel release techniques in comparison to the traditional techniques i.e. adult releases and *Tricho* bit releases. The results are presented in this communication.

MATERIALS AND METHODS

Trichogramma chilonis Ishii used in the present study was obtained from the laboratory culture maintained on *Corcyra cephalonica* (Stainton) eggs. The experiments were conducted in cages ($30cm^3$) wrapped with black muslin cloth on three sides i.e. two lateral sleeves and front door leaving top portion uncovered. The treatments were as follows:

T_1 Adult release method - ten adults / cage were released assuming 50% as female population.

T_2 Tricho bit consisting of 11 parasitised eggs / cage was kept in the cage for parasitoid emergence.

T_3 Tricho capsule consisting of parasitised eggs kept in a 3 x 2 cm cork in which a portion was scoped out and wrapped with nylon mesh (40 mesh size) to allow parasitoids to come out. Tricho capsule was kept in the cage for parasitoid emergence.

T_4 Loose parasitised eggs sprinkled on a piece of cloth.

T_5 Loose parasitised eggs mixed in water and sprayed with the help of atomizer (1 litre).

T_6 Loose parasitised eggs mixed in soap solution (Tween 80 from M/s SD Fine Chemicals, 0.1%) and sprinkled on a piece of cloth. Tween 0.1% solution used in experiment was based on earlier study with other biocontrol agent in which 0.1% solution was found to be optimum (Vyas *et al.*, 1993).

T_7 Loose parasitised eggs mixed in agar solution (M/s SD Fine Chemicals) (1%) and sprayed on to a piece of cloth. Agar solution (0.1%) used in the experiment was based on earlier study with other biocontrol agent in which 0.1% solution was found to be optimum (Dahlan and Grodh, 1997).

T_8 Loose parasitised eggs mixed with talc (M/s Seema Chemicals, Bangalore) were sprinkled on a piece of cloth.

T_9 Loose parasitised eggs mixed with semolina (from open market) sprinkled on a piece of cloth.

T_{10} Loose parasitised eggs mixed with vermiculite (M/s Keltech Energies Ltd., Karnataka) sprinkled on a piece of cloth.

In T_5-T_7, parasitised eggs were mixed with aqueous carriers in the ratio of 1 cc: 20 ml of solution. Eggs along with the aqueous carrier were sprayed on a piece of cloth using an atomizer with nozzle size of 1 mm, which enabled parasitised eggs to come out undamaged. Solid carriers in T_8-T_{10} were mixed with parasitised eggs in 1: 1 ratio and sprinkled on the cloth piece. The cloth pieces of different treatments with the parasitised eggs and the carriers were kept individually in separate cages (i.e. 1piece of cloth per cage) for parasitoid emergence. In $T_2 - T_{10}$, 11 parasitised eggs / cage were kept (considering 95% emergence in the laboratory, 10 adults were obtained with 50% female population). If more eggs were dispensed during sprinkling or spraying, excess eggs were removed with the help of a moist brush. In preliminary studies, egg cards containing 300 UV treated *C. cephalonica* eggs in the ratio of 1female: 60 eggs were hung from the roof of the cage in all the treatments (T_1-T_{10}). The parasitoid: host egg ratio was based on earlier studies (Singh and Jalali, 1994). Fine honey streaks (50%) were provided as adult food. Each treatment was replicated 10 times.

The time taken for release in each treatment was recorded. The per cent emergence was recorded by keeping 50 eggs of each treatment individually for emergence. After emergence, the

eggs sprinkled or sprayed on cloth were observed under microscope to record the eggs with emergence holes. (Each treatment for per cent emergence was replicated 3 times). Per cent parasitisation was recorded after 6 days of release. The eggs that turned black were considered as parasitised. The percentage data were subjected to arcsine transformation before subjecting them to one-way ANOVA.

RESULTS AND DISCUSSION

The time taken for release varied significantly in different techniques of release. In *Tricho* capsule method the time taken for release was 6.4 minutes and it was significantly more than other techniques. Minimum time was required for spraying loose eggs mixed in agar solution and loose eggs in solid carriers like talc, semolina and vermiculite (Table 1). The per cent emergence was significantly higher in the traditional techniques like adult and *Tricho* bit releases, which were on par with some of the newer techniques like release of *Tricho* capsule and loose eggs without carrier, the emergence ranging from 95.0 to 100.0 per cent. However, in the novel techniques where loose eggs were mixed with a solid carrier or aqueous solution, the emergence ranged from 40.5 to 57.1 per cent (Table 1).

Maximum parasitisation was recorded in the treatment where loose eggs was mixed with vermiculite (76.7%) and it was on par with releases of adults (65.9%), loose egg mixed with semolina (61.3%) and loose eggs in agar solution (55.3%), which were significantly higher than other treatments (P = 0.05%). Amongst the aqueous carriers, spraying of loose parasitised eggs in agar solution recorded high parasitisation (55.3%) compared to other treatments where loose eggs were mixed with soap solution or water (Table 2).

Table 1. Per cent emergence and time taken for release in different techniques

Sl. no.	Release method	Time taken (in minutes)	Emergence (%)
1	Adult release	5.0^{b}	100.0 (90.0)^{a}
2	Tricho bit	2.3^{d}	95.0 (77.1)^{b}
3	Tricho capsule	6.4^{a}	95.7 (80.2)^{b}
4	Loose eggs	3.2^{c}	95.0 (77.2)^{b}
5	Loose eggs mixed in water	1.5^{e}	50.0 (45.0)^{c}
6	Loose eggs mixed in Tween 80 (0.1%)	3.4^{c}	52.4 (46.4)^{c}
7	Loose eggs mixed in Agar solution (1%)	1.0^{f}	46.9 (43.2)^{d}
8	Loose eggs mixed in talc	1.0^{f}	40.5 (39.6)^{d}
9	Loose eggs mixed in semolina	1.1^{f}	57.1 (49.1)^{c}
10	Loose eggs mixed in vermiculite	1.3^{e}	57.1 (49.1)^{c}
SEM±		0.1	1.63
CV(%)		5.4	4.74
CD (p=0.05)		0.2	4.86

* Means followed by the similar letters in the columns are not significantly different; The percentage data in parenthesis are arc sine transformed values.

Table 2. Per cent parasitism of *C. cephalonica* eggs in different techniques of *T. chilonis* release

Sl. no.	Release method	Parasitism (%)
1	Adult release	65.9 (55.6)[a]
2	Tricho bit	40.6 (37.0)[b]
3	Tricho capsule	48.5 (43.9)[b]
4	Loose eggs	42.7 (40.2)[b]
5	Loose eggs mixed in water	33.4 (34.1)[b]
6	Loose eggs mixed in Tween 80 (0.1%)	46.7 (42.4)[b]
7	Loose eggs mixed in Agar solution (1%)	55.3 (48.2)[a]
8	Loose eggs mixed in talc	30.4 (31.7)[b]
9	Loose eggs mixed in semolina	61.3 (51.9)[a]
10	Loose eggs mixed in vermiculite	76.7 (61.8)[a]
SEM±		4.94
CV (%)		34.99
CD (p=0.05)		13.93

- Means followed by the similar letters in the columns are not significantly different. The percentage data in parentheses are arcsine transformed values.

Trichogramma are typically released as pupae inside the host egg and eggs are distributed in field just prior to the emergence of adult wasps. *Tricho* bit with parasitised host eggs glued to the cards is the most practical method. Comparison of different techniques of *Trichogramma* release has revealed significant difference in parasitisation between the adult release method and loose eggs mixed with aqueous carriers and in Tricho capsule but not with solid carriers like semolina and vermiculite. The result is contrary to that of *T. galloi* (Pinto *et al.*, 2003), where release of *Tricho* capsule was found superior to broadcasting of adults. The pros and cons of various release techniques were compared. The release of *Tricho* bit card is a simple technique but stapling of the card is not fully understood by farmers, who are tuned to spraying. For experimental purposes, adult release method is practical, but it requires a considerable amount of time and makes large-scale releases impossible. The time required was about 2-4 times more than other techniques. In *Tricho* capsule technique more time was consumed in the preparation but it could provide protection for the parasitised from predators and rain in the field. In this method, the timing of release is also not so critical. However, application of loose parasitised eggs with or without solid carrier had to be done during early morning or evening hours when some amount of moisture is present on the plants, which enables proper sticking of the eggs on the plants. Loose eggs in aqueous carrier could be a good option as parasitised eggs can be sprayed just like insecticides. In the present study, it was observed that eggs did not mix properly with water and spraying solution had to be constantly agitated. However, loose eggs mixed well with soap and agar solution. Spraying of loose eggs gave better parasitisation than in traditional methods like *Tricho* bit release. It is felt that release of *Trichogramma* as loose eggs mixed in solution

(water / Tween / agar solution) and sprayed would be more readily accepted by farmers. The percentage parasitism (33.4 to 55.3%) and adult emergence (46.9 to 52.4%) recorded in this method was significantly lower than the traditional techniques of *Tricho* bit and adult releases. But these factors can be offset by lesser time taken and ease of adoption by farmers. Thus, the present study has indicated that it is possible to develop better alternative methods of *Trichogramma* release both in solid and aqueous carriers. Work on configuration of the spray nozzle for a better droplet size and efficacy for safer discharge of the parasitised eggs would be an important researchable area.

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