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Comparative impact of release frequency of *Trichogramma chilonis* Ishii against *Chilo sacchariphagus indicus* (Kapur) in sugarcane

N. GEETHA[1], ESTHER D. SHEKINAH[2] and P. RAKKIYAPPAN[2]
[1]Division of Crop Protection, [2] Division of Crop Production
Sugarcane Breeding Institute, Coimbatore 641007, Tamil Nadu, India.
E- mail: mvsbi@yahoo.com

ABSTRACT: The egg parasitoid, *Trichogramma chilonis* Ishii, is widely used for the management of the internode borer (INB), *Chilo sacchariphagus indicus* (Kapur), in South India. However, there have been controversies regarding the dosage and frequency of releases required to attain desirable level of INB suppression. In the present study, two on-farm trials were conducted during 2008-09, comparing six releases of *T. chilonis* with continuous releases (from 5 months and 5½ months, respectively in trial I and II to one month prior to harvest) of *T. chilonis* at weekly intervals for their relative impact on INB incidence, intensity, dead hearts, number of nodes and yield, besides juice quality attributes. The cost-benefit ratio of the releases was also worked out. Continuous releases of *T. chilonis* were found to be better than six releases in both the trials. In the first trial, 31.1% and 56.46% reduction in the dead heart incidence was observed with six releases and continuous releases, respectively, while the yields were improved by 10.32% and 25.62%, respectively, with reference to control. In the second trial, continuous releases of *T. chilonis* resulted in significantly lower incidence, intensity, dead hearts and 12.15% increased yield than six releases and control, which were similar. The per cent dead hearts in control was 20.9% and 19.4% in the trial I and II, respectively. The studies also indicated the special benefits from continuous releases when the INB incidence was high. In both the trials, there was a beneficial impact on cane juice quality in terms of significantly improved % polarity and commercial cane sugar (CCS) in continuous releases in relation to control, while in the second trial percentage purity was also found to be significantly enhanced. In two separate assessments on the effect of different grades of INB infestation on the quality, there was a mixed trend in the parameters tested. The yesteryears' work and the present results on the usage of *T. chilonis* with reference to multiple releases for the management of borers are compared and discussed. It is recommended that *in situ* decisions on the frequency of releases are required because even though continuous releases are necessary to maintain the population and damage of INB low, the cost-benefit ratio tilts in favour of six releases in the present study.

KEY WORDS: Sugarcane, *Chilo sacchariphagus indicus, Trichogramma chilonis,* cost-benefit ratio, dosage, frequency, juice quality

INTRODUCTION

Trichogrammatids have been tried and used amid much controversy, against many of the crambid and pyralid borers of sugarcane (Singh, 1994). *Trichogramma chilonis* Ishii is commercially the most viable and commonest species of them. The sugarcane internode borer (INB), *Chilo sacchariphagus indicus* (Kapur), occurs all over India and is a major pest in southern peninsular region. With the advent of spatial and temporal monocropping of sugarcane, the pest occurs throughout the year. Though the pest has a long association with the crop, the sharp increase in the dead heart incidence in the currently popular variety Co86032 has caused enormous concern to the sugarcane growers and sugar factories.

For the management of INB, *T. chilonis* is the lone biological agent that is practically available and employable. In *Trichogramma* usage, often the dosage (Anonymous, 2005), frequency, method of release and distribution (Smith, 1996) have been points of contention, besides the quality of the parasitoid cards produced by the commercial units (Romeis *et al*., 1998). Inundative release of the parasitoid for six times against INB has been the general practice (Kalyanasundaram *et al.,* 1993), though continuous releases had earlier been advocated (Sithanantham *et al.*, 1973). In the present study, we have compared these two methods in terms of pest incidence, intensity, number of internodes produced, yield and quality parameters besides the economics of both the practices. Small scale assessments were also made on the degrees of INB infestation and corresponding quality parameters to determine the impact on sugar recovery.

MATERIALS AND METHODS

The experiments were conducted during 2008-2009 in farmers' fields in two villages, Perumapalayam (I trial) of Athani and Makkalpudur (II trial) of Anthiyur, in Periyar District, Tamil Nadu. In both the trials, the variety was Co 86032 and the crop was drip-irrigated. The age of the crop was five months in the first trial and five and a half months in the second trial. The treatments comprised a) six, weekly releases of the parasitoid from the start of the experiment, and b) continuous weekly releases of the parasitoid from the start of the experiment to one month prior to harvest. A control plot was maintained as check. Each plot was of 0.4 ha size and 20 equidistant spots were selected to be replications within each treatment as well as in control. At each spot, ten canes were marked with black paint for identification and observations were taken on 50 canes in each spot, thus totalling to a sample size of 1000 canes in each plot. In both the methods, *T. chilonis* was released at the dose of 5 cc/ha and distributed equally among the 20 spots in the plot. The parasitoid cards were stapled to the ventral lamina of a green leaf on the cane. In both the trials, during the middle of the experiment period, releases for two weeks could not be made in the continuous releases plot due to lodging of canes caused by heavy winds. Observations were taken on the number of visible internodes (beginning at ground level to the first free internode from the top of cane) per cane, per cent INB incidence (number of bored canes vs. total canes) and per cent INB intensity (number of bored internodes vs. total internodes) at the beginning of the experiment. At harvest, the canes were cut at ground level and detrashed for observations. Besides the above mentioned parameters, the weight of canes per sample (50 canes / replication; 20 replications) and number of dead hearts per sample were taken. Computations were done as follows:

$$\% \text{ incidence} = \frac{\text{No. of canes with INB attack}}{\text{Total no. of canes / sample*}} \times 100$$

$$\% \text{ intensity} = \frac{\text{No. of internodes with bore holes of INB}}{\text{Total no. of internodes in canes / sample}} \times 100$$

$$\% \text{ dead hearts} = \frac{\text{No. of dead hearts due to INB}}{\text{Total no. of canes / sample}} \times 100$$

$$\text{Yield t/ha ** } = \frac{\text{Weight (in Kg) of 50 canes in a sample}}{\text{Total no. of canes / sample x 1000}} \times 1{,}00{,}000$$

* Total number of canes in a sample (replication) is 50 canes and 20 such replications were used.

** The yields are relative figures since they were worked out based on the breeders' assumption that the population of millable canes in the variety Co 86032 is 1,00,000/ha.

The cost-benefit ratios were worked out using the prevailing rates at the time of experimentation by estimating the cost of parasitoid cards (Rs.25/cc), man hours involved in the release of the parasitoid (2 man hrs/release and Rs.100/man day) and the returns in terms of additional yield (Rs.1000/t of cane) accrued in each treatment over control plots.

In these trials, quality parameters of the canes were assessed by drawing a random sample of five canes from every 50 canes sampled in 20 replications in all the three plots. To find out the direct influence of INB infestation on quality, two separate preliminary assessments were made: In the Assessment I, the categories of INB infestation were a) canes with holes caused by INB, b) canes with dead heart caused by INB and c) canes with bunchy tops formed by INB, which are to be compared with canes with no infestation of INB. The sample size was a single cane replicated ten times and was taken from the farm of Sugarcane Breeding Institute, Coimbatore. The variety was Co 86032. In assessment II, the variety, sample size and treatments were similar to the assessment I, but for "canes with dead hearts". The sample was drawn from a farmer's field (var. Co 86032) in the area under M/S Sakthi Sugars, Aappakudal, Tamil Nadu.

The data on incidence were subjected to angular transformation. Statistical analysis was done through one-way ANOVA (Analysis of variance) using SPSS version 11.5 and the treatments were compared by Duncan's Multiple Range Test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Prior to imposing the treatments, the % incidence and intensity of INB were found to be similar among the plots in both the trials. The INB incidence was below 15 per cent in trial I and less than 20 per cent in trial II. The number of internodes was also comparable among treatments in both the trials (Table 1 and 2). At harvest, it was found that the

INB incidence and intensity were higher in the trial II than in the trial I though proportion of dead hearts was not very different (Table 1 and 2).

In trial I, both the release frequencies reduced the population significantly over control, while continuous releases provided greater control than six releases. The respective extent of reduction in INB incidence over control plots was 21.48% and 48.54% (Table 1). A similar trend was observed in the intensity of the pest observed in different treatments. The dead heart incidence, however, was on par in control plots and the "six releases" plots, while in "continuous release" plots it was significantly lower, being 1/3 of what was observed in control.

In trial II, the incidence of INB was generally high and a difference in relative performance of the treatments was observed (Table 2). The per cent dead hearts and incidence did not significantly differ between control plots and the plots with six releases of parasitoid. In contrast, significantly lower incidence of the pest and dead hearts was observed in the plots which had continued releases. The number of internodes per cane did not differ significantly among the plots in trial I, but was significantly different among the treatments in the trial II. It could be inferred that only when the intensity is substantial, it affects the number of internodes produced (Tables 1 and 2). This result is also reflected in the yields obtained. When the intensity levels in control are halved by the parasitoid in "six releases" plots (Trial II, Table 2), though the incidence is on par, the yields differ significantly. In trial I, there was a 10% increase in yield in plots with six releases and at least 25% increase in plots with continuous releases of *T. chilonis* over control. In trial II, the plots with continuous releases had the highest yield, significantly different from the yields in plots with six releases and control plots (Table 2).

However, the cost-benefit ratio was higher and almost equal in trial I and trial II respectively, in the plots with six releases compared to the plots with continuous releases of *T. chilonis*, apparently due to the escalated cost which was not offset by the increase in yields in the latter.

Many factors, *viz.,* climatological variation, management practices, phenotypic characters of the variety and nutrient content of the soil affect the quality and yields of sugarcane and by derivation the degree of INB infestation interacts to increase the impact. Under such circumstances, higher number of releases reducing the INB intensity may have an effect on cane yield and quality. Stalk tunneling by the INB lowers cane yield due to reduced uptake of water and nutrients (David, 1986). A definite strong correlation was found between INB intensity and yields (Seneviratne *et al*., 2001), but it was suggested that the action threshold for intensity level at which the yield is affected, can change from year to year.

The juice quality aspects were found to be affected in varying degrees not necessarily in correspondence to the degree of infestation in the plots (Table 3). Except brix, all other parameters were the best in "continuous releases" plots and significantly different from control at least in one of the two trials, while in the "six release" plots, juice weight, polarity, and CCS were on par with control. The beneficial effect seen on such parameters and thereby on sugar recovery is another indicator of INB management which could not be accounted here in the computation of economics. Brix has a positive correlation with the sugar recovery and the deterioration in juice quality and sucrose losses are also caused by INB infestation, apparently due to the entry of pathogens to the plant and side shooting (David, 1986). The infested canes suffer a significant deterioration in juice quality when the borer damage extends to three or more internodes per cane. Reduction in sucrose content of juice extracted is variable depending on the variety, age of the crop infested and intensity of the attack (David, 1986).

The two independent assessments of INB damage versus the quality, showed a mixed trend. In the first assessment made in the institute farm, there were no apparent effects on the quality parameters except Brix. However, in the second assessment on canes drawn from Periyar District, the impact was more pronounced (Table 4). All the parameters differed between normal and INB affected canes. While these empirical estimates point to the association between INB infestation and juice quality reduction, there is scope for large scale assessments categorizing the infestation aspects further, *i.e*., number of holes per cane, age of the cane, canes with both boreholes and dead hearts, time lapse since dead heart, age of the side shoots formed after dead heart and the associated climatological conditions as factors for a more holistic computation of the loss in quality due to INB.

Inundative releases are practiced in situations wherein successive releases are made so that the parasitoids outnumber the host, so as to subsequently bring the pest population under ETL. In earlier attempts, the period of releases recommended also varied from a few weeks to most of the year (Metcalfe and Breniere, 1969; Mishra *et al*., 1997; Varadharajan, 1976). In sugarcane the suggested frequency of the parasitoid releases has varied over time through trials against different borers. Based on several experiments conducted by the Sugarcane Breeding Institute, the effect of six releases of the parasitoid against INB was not conclusive (Anonymous, 2005), while continuous weekly releases provided consistent positive results (Anonymous, 2006). In trials by other workers on sugarcane borers which occur throughout the season, many releases of *Trichogramma* have been mostly reported to be useful.

Table 1. Field trials on release frequencies of *T. chilonis* for INB management - Trial I (Perumapalayam, Athani)

Treatment	% Incidence	% Intensity	% Dead heart	Internodes NS	Yield kg / 50canes	Yield t ha^{-1}***	Cost: Benefit ratio
Pre- treatment data* NS							
Control	12.8 ± 0.78	2.57 ± 0.31	-	6.55 ± 0.08	-	-	-
Six releases	13.2 ± 1.12	2.35 ± 0.21	-	6.65 ± 0.13	-	-	-
Continuous releases	12.7 ± 0.91	1.89 ± 0.17	-	6.92 ± 0.15	-	-	-
Harvest data**							
Control	68.60 ± 1.25c	4.15 ± 0.15c	20.90 ± 1.91b	25.7 ± 0.33	67.53 ± 3.54a	136.15 ± 3.54a	
Six releases	54.10± 1.21b (-21.14)	2.93 ± 0.17b (-29.40)	14.40 ± 2.36b (-31.10)	27.01 ± 0.52 (+5.10)	75.05 ± 1.17b	150.10 ± 2.34b (+10.32)	1:15.6
Continuous releases	35.30 ± 1.44a (-48.54)	1.65 ± 0.15a (-60.24)	9.10 ± 1.60a (-56.46)	27.17 ± 0.46 (+5.72)	85.45 ± 1.31c	170.90 ± 2.63c (+25.62)	1:10.6

Figures in parentheses are the % decrease or increase over control; * NS- Means of the treatments did not vary significantly by DMRT (P≤ 0.05); ** Means in a column followed by similar letters are not significantly different by DMRT (P≤ 0.05); * Yield (t/ha) is relative as it is calculated by following the formula {weight in Kg of 50canes x 1,00,000}
50 x 1000

Table 2. Field trials on release frequencies of *T. chilonis* for INB management - Trial II (Makkalpudur, Anthiyur)

Treatment	% Incidence	% Intensity	% Dead heart	Internodes NS	Yield kg / 50canes	Yield t ha^{-1}***	Cost: Benefit ratio
Pre- treatment data* NS							
Control	18.5 ± 0.90	1.93 ± 0.16	-	10.65 ± 0.13	-	-	-
Six releases	20.1 ± 1.27	2.49 ± 0.24	-	10.67 ± 0.19	-	-	-
Continuous releases	19.1 ± 1.19	1.96 ± 0.17	-	10.44 ± 0.16	-	-	-
Harvest data**							
Control	77.6 ± 1.39b	6.92 ± 0.28c	19.4 ± 1.26b	24.77 ± 0.37a	64.05 ± 0.72a	128.10 ± 1.45a	
Six releases	71.5 ± 1.43b (-7.86)	3.35 ± 0.16b (-51.59)	18.7 ± 1.22b (-3.61)	27.35 ± 0.52b (+10.34)	68.85 ± 1.67b	137.70 ± 3.33b (+7.49)	1:10.6
Continuous releases	47.2 ± 1.69a (-39.18)	2.64 ± 0.22a (-61.85)	8.7 ± 1.07a (-55.15)	26.94 ± 0.56b (+8.76)	76.2 ± 1.08c	152.70 ± 2.18c (+12.15)	1:10.8

Figures in parentheses are % decrease or increase over control; * NS - Means of the treatments did not vary significantly by DMRT (P≤ 0.05); ** Means in a column followed by similar letters are not significantly different by DMRT (P≤ 0.05); * Yield (t/ha) is relative as it is calculated by following the formula {weight in Kg of 50 canes x 1,00,000}
50 x 1000

Table 3. Pilot assessment of effect of release frequencies on juice quality at trial 1 (Perumapalayam) and trial 2 (Makkalpudur)

Treatment	Juice wt	% juice	Brix NS	% Polarity	% Purity	CCS
Trial 1 (Perumapalayam)		NS		NS		
Control	4.28 ± 0.16a	56.72 ± 0.68	21.40 ± 0.20	19.64 ± 0.21a	91.81 ± 0.51	13.83 ± 0.17a
Six releases	5.11 ± 0.20a	59.07 ± 0.76	21.46 ± 0.19	19.72 ± 0.14ab	91.94 ± 0.45	13.89 ± 0.10ab
Continuous releases	4.46 ± 0.14b	56.96 ± 0.96	21.78 ± 0.13	20.15 ± 0.13b	92.53 ± 0.29	14.24 ± 0.10b
Trial 2 (Makkalpudur)						
Control	3.29 ± 0.19a	46.47 ± 1.35a	21.47 ± 0.13	19.06 ± 0.16a	88.74 ± 0.46a	13.21 ± 0.14a
Six releases	3.75 ± 1.10b	53.40 ± 0.88b	21.30 ± 1.57	19.23 ± 0.16a	90.24 ± 0.54b	13.43 ± 0.14a
Continuous releases	3.69 ± 0.09b	53.76 ± 0.51b	21.79 ± 0.14	19.8 ± 0.14b	90.89 ± 0.34b	13.88 ± 0.12b

* NS- Means of the treatments did not vary significantly by DMRT (P≤ 0.05); ** Means in a column followed by similar letters are not significantly different by DMRT (P≤ 0.05); ***Juice purity is from laboratory tests where extraction per cent is ~50%, hence only relative figures; mean ±SE

Table 4. Quality parameters as influenced by INB infestation: Assessment I and II

Category of canes	Number of canes tested	Corrected Brix	% Polarity NS	% Sucrose NS	%Purity NS
Assessment I					
Normal	10	24.19 ± 0.27b	81.76 ± 1.62	19.32 ± 0.37	79.72 ± 0.91
INB holes	10	24.44 ± 0.37b	82.22 ± 1.91	19.42 ± 0.42	79.42 ± 0.63
Dead heart	10	23.27 ± 0.23a	78.90 ± 1.21	18.74 ± 0.27	80.52 ± 0.77
Bunchy top	10	24.15 ± 0.27b	83.07 ± 1.38	19.65 ± 0.31	81.36 ± 0.61

Category of canes	Number of canes tested	Juice wt. in kg / cane	Corrected Brix	% Sucrose	% Sucrose NS	%Purity NS
Assessment II						
Normal	10	0.42 ± 0.02ab	21.60 ± 0.20b	20.47 ± 0.30b	94.76 ± 0.87b	14.6 ± 0.26b
INB holes	10	0.47 ± 0.02b	20.61 ± 0.47ab	19.03 ± 0.30a	92.48 ± 0.81a	13.43 ± 0.18a
Bunchy top	10	0.38 ± 0.03a	20.43 ± 0.40a	18.94 ± 0.36a	92.70 ± 0.33ab	13.39 ± 0.27a

NS - Treatment means are not significantly different by DMRT (P≤ 0.05); means in a column followed by similar letters are not significantly different by DMRT (P≤ 0.05); mean ± S.E.

Of these, trials on stalk borer, *C. auricilius*, an equivalent of INB in North India, outnumber others and the successful management schedule has twelve releases of the parasitoid (Varma *et al*., 1991; Kaur *et al*., 2008). For *C. infuscatellus* (which occurs only for a short period during early growth) control in contiguous cane fields, releases of *T. chilonis* throughout the year had been suggested (Rao, 1980). In two-year trials on the management of shoot borer and stalk borer, weekly releases of *T. chilonis* @ 50000/ha starting from 21days after planting to 210 days had brought down dead heart and stalk borer incidence (Mishra *et al*., 1997). Adequate control of INB could be obtained by 12 releases of 12500 parasites each / ha which could be reduced to 10 releases if the parasitoid number is 250000/ha (Varadharajan, 1976).

However, in a trial against INB, Kalyanasundaram *et al*. (1993) found six releases of 2.5 cc/ha at 15 days interval from the 4th month onwards to be useful, but the INB incidence even in the control plot was only 37 per cent at the end of the experiment (crop age: seven months). In another set of four field trials evaluating *T. chilonis,* significantly lower INB incidence and higher yields had been recorded in plots that had eight releases of the parasitoid compared to control plots. However, in three of the four trials the incidence even at the end of the release period was either 20 per cent or below even in control plots (Anonymous, 2009). The contradiction here is that INB increases in incidence over time. The intensity of damage is low in the early stages of the crop which increases during the grand growth period (David, 1986). Since that time to harvest, incidences often reach 100% during which time the effect of the parasitoid is more needed. Had there been severe incidence in these experiments as seen in the present studies, the effect could have been otherwise.

On the other hand, as early as 1973, weekly releases albeit at a lower dose (40000 adults/ha) from 4th to 11th month of the crop have been found to reduce the damage by INB (Sithanantham *et al.,* 1973). During these experimentations, the pest incidence had been to the tune of 73.6% in control plots which is almost as in the present second trial. However, since these different trials tend to differ in doses of parasitoid, timings, and frequencies, the effectiveness of the releases as such cannot be compared because of the varying levels of pest incidence during the period of experimentation at different locations. In the present study, the two different frequencies of parasitoid have been applied in the same field and this comparison has brought out the subtle difference in efficacy due to the number of releases as seen in the INB incidence and intensity. Further, if there is an additional stress to the crop due to diseases and moisture stress, the effect of INB will be more pronounced and that is where the continued releases help in prolonged protection. Since the cost factor is tilting in favour of lower doses under normal conditions (as in the present study), the farmer has to decide *in situ*. Probably if the incidence in a particular field is high, the farmer would have to go for more releases. Or, on a prophylactive basis he would have to assume the population explosion based on initial incidence, variety and the past history. Further, any unfavourable environmental effects and variations in the quality of certain batches of the parasitoid can affect the field efficacy and the continuous releases also mitigate these eventualities to guarantee consistent performance in the INB management.

In our earlier experiments done at SBI, releasing *T. chilonis* a few times did not give consistent results and the later trials done with continuous releases were found to have superior control efficacy (Anonymous, 2007). Continuous releases @2cc/ac at fortnightly intervals also did not provide consistent positive results (Anonymous, 2005) and thus weekly releases were reckoned to be necessary.

The view that many releases of *T. chilonis* may not be feasible is incorrect, as proven by the 12 releases being made for stalk borer control in north India which is practically in operation. Similarly, INB damage being cumulative in nature, releases of the parasitoid can be delayed depending on the incidence until the 6th month or 7th month but not later and has to be continued to a month before harvest. Otherwise, with the increase in the recent pattern of attack, *i.e.* dead hearts, the infestation would result in more of dead hearts and a greater loss in yield. Continuous releases from the 5th month or 7th month did not show a difference in performance, but releases from the 9th month were not found useful against INB (Anonymous, 2005).

The option of six releases may work out as more attractive in cases where population/incidence tends to be low or where the variety is naturally tolerant to INB damage. In these cases, it is preferable to delay the releases rather than to start and finish the releases during the early period of cane forming season. However, continued releases are now shown to be clearly better than six releases in bringing down the pest problem, especially when the incidence levels are higher, obviously by assuring the parasitoid availability throughout the pest occurrence period. Further, the present studies have also shown beyond doubt that both the release systems provide attractive cost-benefit ratios and in that aspect six releases are equal to or better than continuous releases. Thus, under normal or low incidence levels, six releases should be sufficient.

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