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A NEW RECORD ON MASS REARING OF PUPAL PARASITOID, *TETRASTICHUS HOWARDI* (OLLIFF) USING SILK WORM PUPAE FOR THE MANAGEMENT OF SUGARCANE STEM BORERS IN SOUTH INDIA

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ABSTRACT

The sugarcane internode borer, *Chilo Sacchariphagus indica* (Fabr.) (Lepidoptera: Pyralidae) spends the largest part of its life cycle inside the cane, which provides protection against the action of conventional control methods. Biological control has been considered a viable and an alternate to control method to this pest in sugarcane, *Saccharum officinarum*. This paper reports that an augmentative release of an entoparasitoid, *Tetrastichus howardi* for the management of sugarcane internode borer is popularized among the sugar industries in south India. Mass rearing technology of *T. howardi* is standardized in pupae of ERI (*Philosomia ricini*) and Mulberry silk worm (*Bombyx mori).* A single pupa of *B. mori* is able to produce average number of 115 tetrastichus offspring. The life cycle of the parasitoid was around 22 days. The presence of the parasitoid in India opens a new perspective on suppression of the sugarcane stem borers. The insect is well adapted to the laboratory and field conditions, which can be mass produced in large scale by using eri and mulberry silk worm pupae and may, became an additional option for the integrated pest management against various lepidopteron insect pests.

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INTRODUCTION

The Biocontrol agent, *Tetrastichus howardi* (Olliff) (Hymenoptera; Chalcidoidea: Eulophidae) is a gregarious pupal parasitoid which has been recorded as a primary parasitoid or facultative hyper parasitoid associated with a great number of Lepidoptera pest species of important crops [1-2] The parasitoid was introduced in South Africa for the control of two key Lepidoptera pests, the borers *C. partellus* and *Busseola fusca* both species being severe pests of corn and sorghum in that country [3-4]. Recently, recorded that the sugarcane stem borer species *Diatraea saccharalis* is a major pest in the Americas [5-6] is capable of causing loss in biomass, death of the apical meristem and reduction in sugar and alcohol production [7-8]. *Diatraea saccharalis* caterpillars develop inside the sugarcane stalks, which diminishes the efficacy of the insecticides used to control them [9]; hence the interest is created in the use of biological control agents to suppress *D. saccharalis* infestations [10]. Hymenopteran parasitoids are frequently used as natural enemies in numerous applied biological control programs targeting *D. saccharalis* [11].

Tetrastichus howardi parasitized the fifth instar *D. saccharalis* larvae and emerged in the pupal stage. Also *T. howardi* parasitized young *D. saccharalis* pupae and emerged from the adults. This is the first time that this pattern of behavior and development of *T. howardi* has been reported. Parasitism of the different biological stages of *D. saccharalis* by *T. howardi* revealed the ability of this natural enemy to regulate the development of various sugarcane borer life stages, and this can be attributed the longer life span of the adult stage of *T. howardi* [12] compared with that of the egg parasitoids *Trichogramma*

spp. (Hymenoptera: Trichogrammatidae) [13-14], thé larval parasitoid *C. flavipes* [7]. Thus, in sugarcane fields, this eulophid has more time than other studied parasitoid species to locate and parasitize its host.

Tetrastichus howardi displays high plasticity by parasitizing pupae of Chilo species in sugarcane associated pests. However, further studies on the cell biology, physiology and ecology of this parasitoid are necessary to better understand the parasitoid-host relationships and to increase the chances of success of this important natural enemy in biological control programs. The parasitoid *Tetrastichus howardi* Olliff (Hymenoptera: Eulophidae) has been recorded parasitizing the pupae of several lepidopteran families, including: Crambidae, Noctuidae and Plutellidae [15-16] and it has been used to effectively control several lepidopteron pests. *Tetrastichus howardi* was also found parasitizing *D. saccharalis* larvae, which motivated this study on the parasitism of *T. howardi* on various life stages of this important sugarcane pest. The aim of this study was to evaluate the biological characteristics of *T. howardi* parasitizing mulberry silk worm, *Bombyx mori* for mass production purpose to control sugarcane stem borer.

Routine surveys in sugarcane fields were conducted in parry command area of Tamil nadu and Pondicherry states, India. The species *C. sacchariphagus* were collected at different phases of development, during November 2007, resulting in two pupae which were parasitized and further it was mass reared in the laboratory. The specimens of the parasitoid were identified by Dra. Angélica M. Penteado-Dias and Dr. John La Salle as *Tetrastichus howardi* (Olliff) (Hymenoptera; Chalcidoidea: Eulophidae). Since then, culture of this species has been maintained on pupae of *Spodoptera litura* (Lepidoptera: Noctuidae) and mass producing and frequent releases is being practiced in our EID Parry (I) Ltd, sugar command areas for the management of sugarcane stem borer, *C. sacchariphagus indica.*

MATERIALS AND METHOD

Description of the parasitoid

Tetrastichus is a gregarious internal pupal parasitoid. The small insect having 1-3 mm size with 4-segmented tarsi and are diverse. They are parasitoids with a wide variety of hosts belonging to order *Lepidoptera* and *Diptera*. Some are egg parasitoids, and few are hyper parasitoids. *Tetrastichus* is a gregarious internal pupal parasitoid. The adult wasp is 3 mm long, with a dark metallic blue thorax and a black abdomen with a faint metallic luster completing its life cycle in about 17 days. The females apart from parasitizing the pupa also destroy many eggs of Lepidopteron order by host feeding. This is considered an important factor in reducing the pest population. Because of its higher reproductive capacity coupled with the parasitizing efficiency, the parasitoid was thought of as an effective biological control agent. With this background, the parasitoid was sampled for its occurrence in sugarcane command area of Nellikuppam. They were then field collected and laboratory reared on the larvae of internode borer. After laboratory rearing they were tested for pilot scale production and when found feasible the parasitoid was subjected to laboratory and field testing. A field experiment to assess the efficiency of the released *Tetrastichus i*n the field was conducted in the sugarcane command area of E.I.D.Parry (I) Ltd., Nellikuppam and Pudukottai during the year 2003-04 with the objective of to conduct a field trial for determining the field efficiency of the *Tetrastichus* when populations of 1500 adults are released per acre.

Collection of various host insects for the study

The experiments were conducted in entomology lab at EID Parry (I) Ltd., Pugalur Unit during 2012 to 2016, and test insects of *Helicoverpa armigera*, *Spodoptera litura*, *Chilo partellus*, Cockroach and House fly were reared in the respective artificial diet in the laboratory, Oozy fly, *Philosomia ricini* and Bombyx mori were collected in the farmers who is rearing silk worm cocoon in our local area. The sugarcane internode borer, *Chilo sacchariphagus indica* was collected in the sugarcane field and used fro the experiments.

Back ground of the study.

Field trials to test the efficiency of the released adults were conducted at 1500 adults per acre in the West division of Nellikuppam command area and Viralimalai division of Pudukkottai command area during 2003-04. The release of the parasitoid were made at 1500 adults per acre in three splits at 30 days interval after dividing the one acre, to facilitate the easy release of the parasitoid. Simultaneously a check plot was maintained separated by a distance of at least 400-500 meter from the release plot. After release ,the observation on the incidence of internode borer were recorded by selecting 10 stools at random and at each spots 50 stalks were counted for healthy and infested one to calculate the percent incidence of the borers. Table: 6 showed that the results on the efficiency of the released *Tetrastichus howardi* and the table it is evident that the released parasitoid is capable of reducing the internode incidence between percent and thus an average of 49.28% (Table: 6). Thus from all the above findings it could be thus inferred that *Tetrastichus* is an effective pupal parasitoid against the borer pests of sugarcane and is capable of reducing the borer population between 34.98% and 64.41%

Mass production of *Tetrastichus howardi*

The biocontrol agent, *Tetrastichus howardi* was initially mass reared using pupae of tobacco cut worm, *Spodoptera litura* in our biocontrol lab at Pugalur and Nellikuppam Sugar Mills. The host insect, *S. litura* young stage larvae from 1st to 3rd instars were fed with castor leaves in a group and the later stages 4th and 5th instars were reared separately on artificial diet prepared by using basin

flour, yeast, and multivitamins sub suiting agar as a wetting agent to the media. The fully grown pupa of *S. litura* was used to mass produce the parasitic insect, *Tetrastichus howardi.* Later, the works have initiate in search of an another host insect to alter the existing host insect, tobacco cut worm, *S. litura* to mass produce *T. howardi* to increase the percentage of cane area to cover from 80 to 100%. Ultimately it is an urgent need of finding new host insect which is bigger in size and rich nutrients supporting the growth and production of more offspring. *Tetrastichus howardi* is a pupal parasitoid for all the lepidopteron insects ich needs particularly pupal stage to continue its life cycle and its progeny development. In the study, initially we have tried few locally available lepidopteron insects pupa like; cotton boll worm, *Helicoverpa armigera*, Pink boll worm, *Sesamia inference* reared in artificial diet and eri silkworm, *Philosamia ricini* and Mulberry silk worm, *Bombyx mori* reared on castor leaves as referred by Priyadarshini [17] in the biocontrol laboratory at Pugalur Sugar Mill. Pupa of *Bombyx mori and Philosomia ricini* was able to parasitize by the tetrastichus insects and its progeny was recorded more than the progeny produced by *S. litura*. The pupal size of *B. mori and P. ricini* was more than the other insects which was varying from 2.0 to 2.5 g wt, however the percent parasitization by Tetrastichus insects of pupa of *Bombyx mori* recorded only 10% and it was noticed that the most of the pupae infecting with microorganisms however the larva and pupal cultures of *Philosomia ricini* could able to multiply well in the laboratory at temp., vary from 25 to 27 °c without any mortality. However, the pupae of *Bombyx mori* and *Philosomia ricini* showed parasitizing more than 50% by Tetrastichus adults and considerable number of progeny was developed in the laboratory. Hence the tetrastichus is being multiplied continuously on pupae of both *Bombyx mori* and *Philosomia ricini* and the same is being released to our sugarcane cane command areas for the management of sugarcane internode borer.

Biocontrol agents release plan:

To make this biocontrol agent release model for more sustainable and familiarize among the farmers, unique distribution system and the payment facilities are routed through company fortnight interval. Parry is a pioneer organization to introduce this rural entrepreneur model for biocontrol agent release which is now been followed by other sugar mills in India and the same is also following by other foreign countries. The pupal parasitoid, *Tetrastichus howardi* were sequent release @ 500 insects/month on 5th, 6th and 7th month aged cane for 3 months. Biocontrol agent released and non-released plots data was obtained from our net based Cane Management system (CMS) for the sugar year 2012-15 in all the sugar Mills and was statistically analyzed with One Way ANOVA to draw the conclusion.

RESULTS AND DISCUSSION

Host insect studies

The parasitoid *Tetrastichus howardi* Olliff (Hymenoptera: Eulophidae) has been recorded from the pupae of several lepidopteron families, including: Crambidae, Noctuidae and Plutellidae [18] and it has been used to effectively control several lepidopteron pests which motivated in the present study on the parasitism of *T. howardi* on various crop pests including some of the important sugarcane pests. In the present study tested with the pupal parasitoid, *Tetrastichus howardi* is able to parasitize many species of insects pupae *viz*., cockroach, house fly, Oozy fly, *Helicoverpa armigera*, *Spodoptera litura*, Philosomia ricini, *Chilo partellus*, *Chilo infuscatellus* and *Chilo sacchariphagus indica* in the laboratory. Experiments were performed in the Laboratory of Entomology at EID Parry (I) Ltd., Pugalur Unit, which has shown the potential to be used in the biological control program to manage the various agriculturally important pests. The parasitism intervals were as follows: 48 h for all the host pupae and it developmental stages were incubated 288 h to emergence of its progeny from the pupa. Result of the study revealed that the pupae of ERI silk worm and Mulberry silk worm produces highest progeny an average yield of 125 and 115 adults/pupa respectively (Table-1) and the life cycle (egg-adult) durations of *T. howardi* in pupae of host insects varied from 12.00 ± 3.00 days among the insects. Vargas *et al* [12] reported the similar findings that the parasitism of the different biological stages of *D. saccharalis* by *T. howardi* revealed the ability of this natural enemy to regulate the development of various sugarcane borer life stages, and this can be attributed the longer life span of the adult stage of *T. howardi.*

Biology of tetrastichus developed in Mulberry pupa

Tetrastichus is a gregarious internal pupal parasitoid. The adult wasp is 3mm long, with a dark metallic blue thorax and a black abdomen with a faint metallic luster completing its life cycle in about 18 days. Each female lay up to 100-150 eggs in a pupa. The eggs after development feed on the internal content and develop inside the pupae. The development is completed in 15- 18 days where the adults emerge. Thus it takes 18 days to complete one cycle. There are several generations each season, usually twice the numbers of annual generations of its host. The females apart from parasitizing the pupae also destroy many eggs of Lepidopteron order by host feeding. This is considered an important factor in reducing the pest population. Apart from the above factor, the high fecundity of the female is considered as an important attribute in biological control.. The hypothesis that the fecundity of parasitoids is correlated positively with their ability to suppress host populations is supported by data exclusively from the order *Lepidoptera* as per La Salle and Polaszek [19]. Because of its higher reproductive capacity coupled with the parasitizing efficiency, the parasitoid was thought of as an effective biological control agent. With this background, the parasitoid was sampled for its occurrence

in sugarcane command area of Nellikuppam. They were then field collected and laboratory reared on the larvae of internode borer. After laboratory rearing they were tested for pilot scale production and when found feasible the parasitoid was subjected to laboratory and field testing.

The life history and development of Tetrastichus parasitizing silk worm pupae were studied. The mean lifetime fecundity was 95 with a maximum of 150, and the proportion of female progeny averaged 90%. A male and a female lived for an average of 20 to 22 and 22 -26 days, respectively. The duration of development for each stage was as follows: egg, 2 days; the 1st instar larva, 2 ± 1 days; the 2nd instar larva 2 ± 1 days; the 3rd instar larva 3 ± 1 days; the 4th instar larva, 6 ± 2 days; and the pupa, 8± 2 days Total developmental duration from hatching of the larva to adult emergence required 12 ± 2 days, and female development took 1- 2 days more than that of males in the room temperature of 27 ^{0}C and 60 to 70% RH. Oliveira et al. [14] also reported that the similar findings in the study. Experiments were conducted to develop methods of mass rearing the parasitoid, *T. howardi* on ERI and Mulberry silk worms pupae aged 4 to 8 days were parasitized and there was no statistical difference between these ages. 6 days aged pupae were standardized to parasitize. The average number of offspring's was 90 females and 25 males per pupa. Parasitization of less than two day old and between 1-3 days old could not parasitize properly. Each pupa had parasitized by 8 female and the ratio of copulation between females and males was 1:1 ratio. An average number of offspring's was recorded and varied from 75 to 90 females and 75 to 60 males per pupa. The illustration of various developmental stages of tetrastichus offspring's in the parasitized pupae of mulberry silk worm shown in the Figs., 1 & 2. It is concluded that to achieve the mass production of pupal parasitoid, tetrastichus is requires big sized pupa to get more number of progeny to cover larger area of crop at a time.

Determining the field efficiency *Tetrastichus howardi*

Field experimental trials conducted to test the efficiency of tetrastichus releasing @ 1500 adults per/ac in the West division of Nellikuppam Unit command area and Viralimalai division of Pudukkottai Unit command area during 2003-04. Result of the study showed that the efficiency stem borer damage was drastic ally reduced in the *Tetrastichus howardi* released plots than the non released plots, the table it is evident that the released parasitoid is capable of reducing the internode incidence between percent and thus an average of 49.28% (Table- 2). Thus from all the above findings it could be thus inferred that *Tetrastichus* is an effective pupal parasitoid against the borer pests of sugarcane and is capable of reducing the borer population between 34.98% and 64.41%. The parasitic insects should be released at the rate of 1500 adults/ac starting from 150 to 210 days after planting. The 1500 tetrastichus adults should be released @ 500 adults/ac/month split into three intervals from 150 days after planting till 210 DAP. The recommendation is purely based on the efficiency of the released parasitoid in the field and its effect on the subsequent borer population. It could be observed that three months after releases were made the borer population has drastically come down in all the release plots. Release to be made either in morning or evening hours. Chemical pesticide should not be sprayed in the released field.

Table 1. **Host insects tested for the development of *Tetrastichus howardi* in the lab.**

S. No	Host insects	**Offspring development** (Avg nos.of tetrastichus/pupa)
1	Cockroach	70
2	Oozy fly	55
3	*Helicoverpa armigera*	70
4	*Spodoptera litura*	65
5	*Philosomia ricini*	125
6	*Chilo partellus*	55
7	*Chilo infuscatellus*	70
8	*Chilo sacchariphagus indica*	65
9	*Bombyx mori*	115

Table 2. **Field efficiency of pupal parasitoid, *T. howardi* tested in EID parry Mill command areas.**

Exp details	Rep	Tetrastichus released/ac (nos)	INB % Incidence			INB % reduction over untreated plots
			Treated plots		Untreated	
			Initial read	Final read	control plots	
Exp-1 (Viralimalai)	rep-1	1500	3.3	12.8	24	46.67
	rep-2	1500	4.7	9.5	26.7	64.41
	rep-3	1500	6.8	10.9	27.3	60.07
Exp-2 (West)	rep-1	1500	11.5	18.4	28.3	34.98
	rep-2	1500	16.1	19.2	33.6	42.86
	rep-3	1500	12.3	16.9	31.7	46.68
Average (%) =						49.28

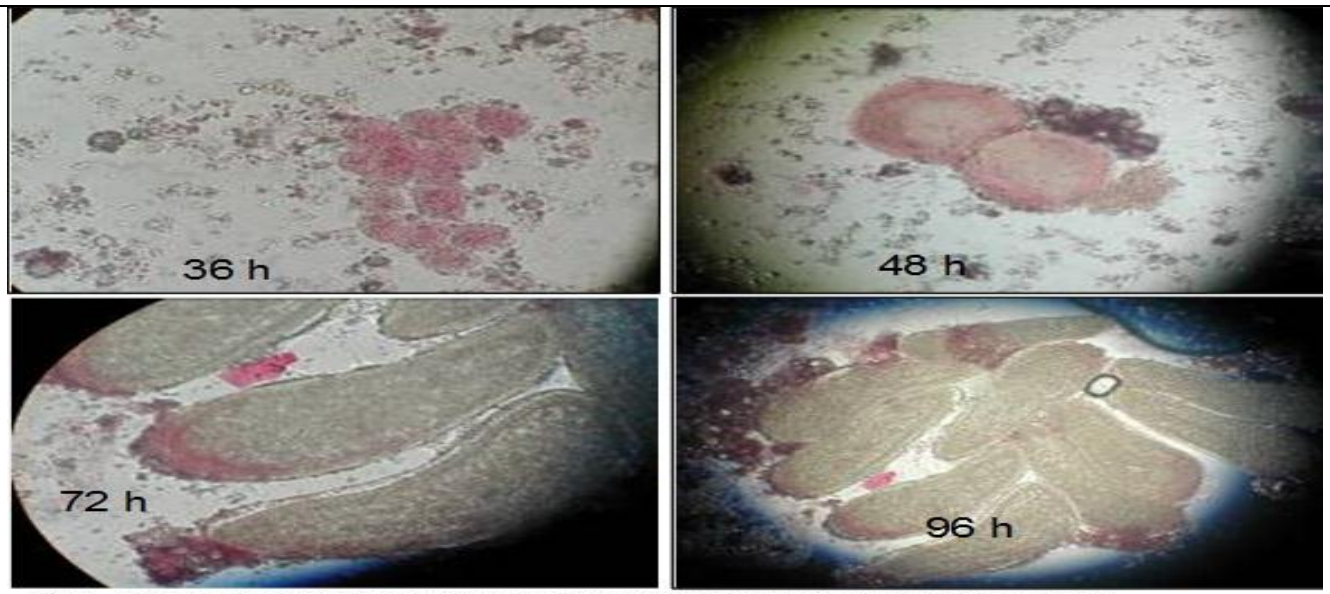

Fig:1. Developmental stages (36 h to 96h) of tetrastichus in *Bombyx mori* pupa

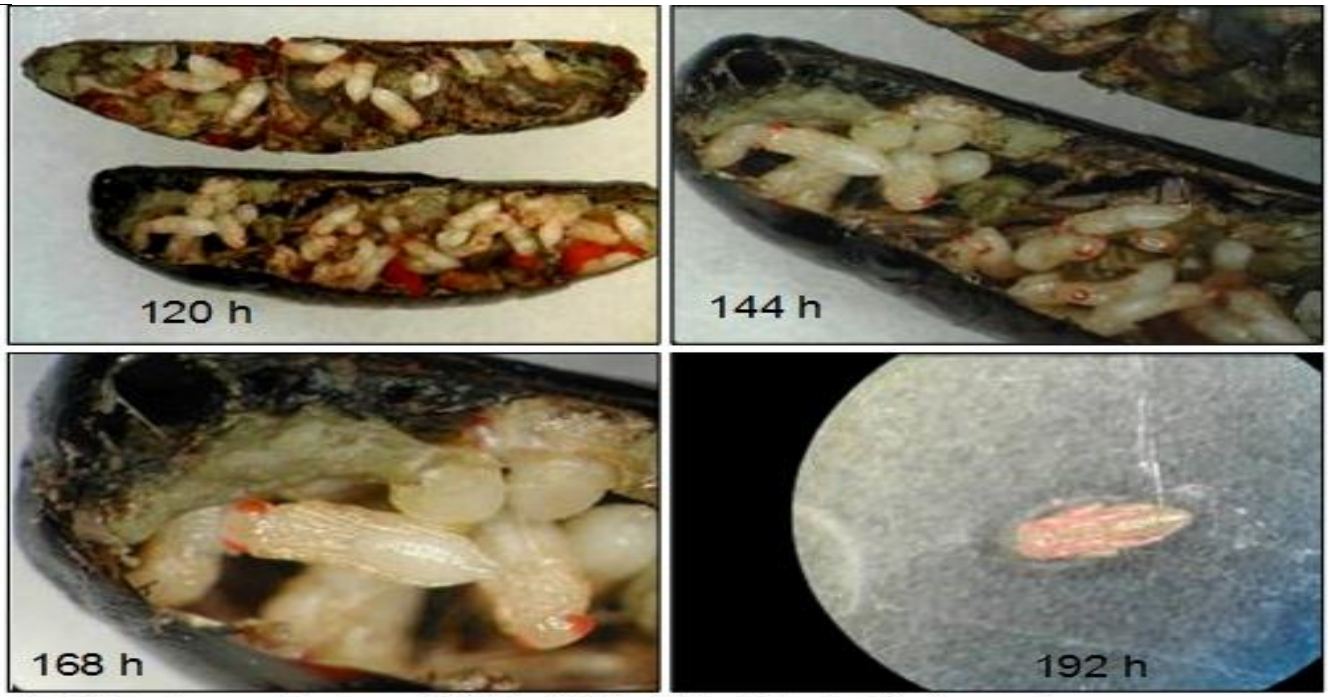

Fig:2. Developmental stages (120 h to 192h) of tetrastichus in *Bombyx mori* pupa

CONCLUSION

Our results indicate the existence of a natural enemy (*T. howardi*) which parasitizes all the lepidopteron pupae. Its potential use should, however, be investigated for improvement in the method of mass rearing, the dispersion in the field and the association with other parasitoids of eggs (*Trichogramma galloi*) and larvae (*Cotesia flavipes*) applied at the same time. The pupal parasitoid, *Tetrastichus howardi* parasitizes the pupae of *C. sacariphagus* indica in sugarcane and therefore seems to be a suitable candidate for the biological control against sugarcane internode borer and other industries where this stem borer is an important pest. The mulberry silk worm cocoon is highly supportive to utilize for mass rearing of this natural enemy for INB management and also commercial use in sugarcane industries.

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