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Studies on reproductive efficiency and biometric parameters of progeny adults of *Nesolynx thymus* (Girault) (Hymenoptera: Eulophidae) – a parasitoid of *Exorista bombycis* (louis) (Diptera: Tachinidae) subjected to higher temperature

S. H. Divya[1] and K. Sathya Prasad[2]*

[1]*Department of Sericulture, Yuvaraja's College, University of Mysore, Mysuru-570 005*
[2]*Pest management laboratory, Central Sericultural Research and Training Institute, Srirampura, Mysore-570 008.*

Email: divyash2003@gmail.com

ABSTRACT

Of the various factors, which influence the developmental characteristics of the parasitoid, the occurrence of high degree of sensitivity to temperature among the parasitoid is considered to be one of the drawbacks in biological control programmes of the pests using parasitoids. In the backdrop of this, initially, an attempt was made know whether Nesolynx thymus an indigenous, gregarious ecto-pupal parasitoid, would complete its life cycle at temperatures between 30 and 35°C. Later at the upper vital temperature limit for development, the female parasitoid was maintained for 10 generations to induce temperature tolerance. It was observed that the parasitoid could successfully complete its life cycle upto 32°C. No significant decline in rate of parasitization was observed upto 8th generation. Though there was no consistency in the progeny production at different generation, some decline was observed from 8th generation onwards. While, the parasitoid sex ratio remained unchanged. Further, The study was continued for 10 generations and the biometric characters 1 to 10 generations an increase in the body length, head width, wingspan of male and female and abdomen width and length of females. However, in the initial 5 generations, these parameters were significantly lower compare to control and thereafter, subsequent generations, they are significantly higher over the control. The results are discussed in relation to the induction of temperature tolerance in N. thymus and stabilization in the parasitoid progeny production.

Key words: *Exorista bombycis*, ecto-pupal parasitoid, Nesolynx thymus, biometric characters, developmental temperature

INTRODUCTION

The use of biocontrol agents has become a successful venture in several fields for the management of pests. Altogether, twenty parasitoids have been reported to parasitise the uzi fly (Kumar *et al*., 1992). Among them, *Nesolynx thymus* has been identified as a potential parasitoids of *E. bombycis* owing to its possession of desirable attributes of a good biocontrol agent like efficient host searching ability, synchronization of its life cycle with that of its host, high degree of host specificity, adaptability to the host habitat, amenability to laboratory multiplication, etc. (Kumar *et al*., 1992).

Nesolynx thymus is an indigenous, gregarious, ecto-pupal parasitoid which forms an important components of IPM against the uzi fly, *E. bombycis* which causes 10-20% loss to silkworm crops (Kumar *et al.*, 1993; Sathya Prasad *et al.*, 2006).

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N. thymus has been recommended as a biocontrol agent of *E. bombycis*, apart from exploiting it as one of the components of an IPM package developed by the Central Sericulture Research and Training Institute (CSR&TI), Mysore, against uzi fly, which also comprises the use of a nylon net / wire mesh enclosure and an ovicide (uzicide) (Kumar *et al.,* 1993) and an adult attractant (uzi trap) (Sathya Prasad *et al.,* 2006).

Of the various factors, which influence the developmental characteristics of the parasitoid, the occurrence of high degree of sensitivity to temperature among the parasitoid is considered to be one of the drawbacks in biological control programmes of the pests using parasitoids. In the back drop of this an attempt was made to induce temperature tolerance in *N. thymus* by exposing them to temperatures ranging from 30 to 34°C as the investigation of the previous chapter has clearly indicated no development at a very low temperature of 10 and higher of 35° C (Table1) Hence, the insects were continuously expose to temperatures ranging from 31 to 35°C and it was observed that the parasitoids could successfully complete their life cycle at 32° C beyond which no development was recorded. Hence an attempt was made to collect the population developed on *E. bombycis* pupa at 32° C and period to the host pupa and maintain at 32° C temperature and relative humidity 60±5%.

MATERIAL AND METHODS

The Nucleus cultures of *N. thymus* were procured from the insectary of Pest Management Laboratory (PML), Central Sericultural Research and Training Institute (CSR&TI), Mysore and reared on uzi pupa following the methods suggested by Kumar *et al.,* 1996. The experiment repeated thrice with 5 replications.

To study the induce temperature tolerance in the parasitoid by exposing the 2 day old females of *N. thymus* adults was provided 2 day old *E. bombycis* pupae for parasitisation at a parasitoid host ratio of 1 : 5 and kept in an incubator at constant temperature 32º C. The pupae were allowed to stay with *N. thymus* for a period of 2 days. During the period of oviposition, an aqueous honey solution at 50% smeared on a strip of paraffin paper was provided as parasitoid adult food. After 2nd day, the parasitoid adults were separated from the host pupae and the latter were kept for parasitoid development at the same temperature. After the emergence of the parasitoid, the parameters such as percent parasitization, developmental duration, brood allocation, progeny production ,sex ratio and adult longevity (with diet) were recorded.

To understand impact of reproductive performance of 1st generation N thymus adults, the adults recovered from uzi pupae in parent generation, were provided 2 day-old randomly selected *E. bombycis* pupae at a parasitoid-host ratio of 1:5 for parasitization.

To understand impact of biometrical attributes of the parasitoid of parent generation adults, 10 each of male and female adults were anaesthetized using chloroform and the parameters are body length, head width and wingspan of males and females and abdomen length and width of females were measured using ocular microscope at 18 X.

The experiment was continued the surviving progeny in each generation will be further exposed to temperature stress till stabilization in parasitization and survival is achieved.

The results were subjected to one way analysis of variance (ANOVA) followed by Duncan Multiple Range Test (DMRT) for the level of significance.

RESULTS

Results on the progeny production by *N. thymus* at 32ºC up to 10 generations on *E. bombycis* pupae are furnished in table-1.

Per cent parasitization:

The mean values for the parameter ranged from 40.00±3.16 (generation 10) to 72.00±4.90 % (generations 1,2 and 4). The parameter was significantly higher (P≤0.01) and statistically similar from generations 1 to generation 8 (64.00±4.00) and it decreased drastically later. The per cent parasitization in control was *at per* with that at generations 1 to 8.

Developmental duration:

The parameter varied from 14.00±0.32 (generations 7 and 10) to 15.00±0.32 days (generations 3). The parameter was significantly higher (P≤0.01) at control (15.67±0.33 days) than the treatments where the values were statistically *at par*.

Progeny production

Male:

Significantly higher (P≤0.01) mean values for the male progeny production were recorded in control (8.40±0.93) whereas significantly lesser value at generation 5 (3.20±0.58). The parameter showed decreasing trend except at generations 5.

Female:

The values for the parameter ranged from 75.80±12.00 (generation 10) to 173.60±8.44 (generation 1). It showed decreasing trend but with significantly higher values at generation 1 and control (189.20±17.16) and lesser values at generations 8 (81.20±10.41) and 10.

Total: The total progeny production varied from 79.60±11.96 (generation 10) to 179.80±9.12 (generation 1). The parameter showed decreasing trend but with significantly higher values at generation 1 and control (197.60±18.08) and decreased values at generations 8 (84.80±11.08) and generation 10.

Sex ratio:

Table-1. Reproductive efficiency of *Nesolynx thymus* when allowed to parasitize *Exorista bombycis* at 32ºC for 10 generation

Generation (No.)	Percent parasitization@	Developmental duration (days)	Progeny production (No.)			Sex ratio (M:F)
			Male	Female	Total	
1	72.00±4.90 [a]	14.60±0.24 [bc]	6.20±0.86 [abc]	173.60±8.44 [a]	179.80±9.12 [ab]	1:29.67±3.26
2	72.00±4.90 [a]	14.80±0.20 [bc]	6.80±1.16 [ab]	157.60±13.58 [ab]	164.40±14.29 [ab]	1:25.16±3.52
3	68.00±4.90 [a]	15.00±0.32 [ab]	3.80±0.66 [bc]	106.60±11.76 [cd]	110.40±11.93 [de]	1:30.90±5.32
4	72.00±4.90 [a]	14.60±0.24 [bc]	6.40±1.54 [abc]	154.40±3.49 [ab]	160.80±4.84 [abc]	1:29.87±6.26
5	68.00±4.90 [a]	14.80±0.20 [bc]	3.20±0.58 [c]	102.00±9.18 [cd]	105.20±9.46 [de]	1:35.74±6.59
6	64.00±4.00 [a]	14.20±0.20 [bc]	6.40±1.50 [abc]	137.00±12.31 [bc]	143.40±12.50 [bcd]	1:26.67±6.25
7	60.00±3.16 [ab]	14.00±0.32 [c]	5.60±0.93 [abc]	119.00±12.95 [c]	124.60±13.58 [cd]	1:22.31±2.66
8	64.00±4.00 [a]	14.40±0.40 [bc]	3.60±0.68 [bc]	81.20±10.41 [d]	84.80±11.08 [e]	1:23.63±1.66
9	44.00±4.00[c]	14.20±0.20 [bc]	4.40±1.29 [bc]	105.20±12.58 [cd]	109.60±13.79 [de]	1:28.91±4.54
10	40.00±3.16 [c]	14.00±0.32 [c]	3.80±0.37 [bc]	75.80±12.00 [d]	79.60±11.96 [e]	1:20.96±4.23
Control	72.00±4.90 [a]	15.67±0.33 [a]	8.40±0.93 [a]	189.20±17.16 [a]	197.60±18.08 [a]	1:23.26±0.57
F value	7.411 **	2.645 **	2.667 **	10.257 **	10.107 **	0.982 NS

Values given in the Table are the mean of 5 replications (Mean ± SE);
Mean values followed by the same superscript in columns are statistically not significant;
@ - Out of 5 pupae provided for parasitization;
** - Significant at 1 %;NS – Nonsignificant;

The values for male to female ratio among the different generations ranged from 1:20.96±4.23 (generation 10) to 1:35.74↑16.59 (generation 5). Though the variation existed among the treatments, it was not significant statistically.

Results with respect to the effect of 32ºC temperature on the biometrical characteristics of *N. thymus* at up to 10 generations on *E. bombycis* pupae are furnished.

Body length

Male:

The male body length fluctuated between 1.560±0.020 mm (generation 3) to 1.848±0.015 mm (generation 8) among the treatments. The parameter at generations 8 to 10 (1.836±0.016 mm) was statistically similar and higher than the remaining treatments. The parameter showed increasing trend with increase in generations.

Female:

The female body length ranged from 2.064±0.010 mm (generation 5) to 2.214±0.019 mm (generation 9). The mean values for the parameter at generations 8 (2.117±0.028 mm) to 10 (2.196±0.024 mm) were statistically *at par* and significantly greater (P≤0.01) than the remaining treatments. The parameter showed increasing trend with increasing generations.

Head width

Male:

The parameter for the parasitoid males ranged from 0.384±0.010 (generation-2) to 0.558±0.009 mm (generation 9). The latte treatments revealed significantly higher (P≤0.01) value among the treatments. The values for the parameter recorded from generations 6 (0.540±0.013 mm), generation 8, 10 (0.552±0.008 mm) and 9 were statistically comparable.

Female:

The head width of the parasitoid females was statistically similar in generations 6 (0.648±0.012 mm), 8, 10 (0.654±10.019 mm) and 9 (0.660±0.015 mm) among the treatments. It was significantly greater (P≤0.01) at generation 9 than that at the remaining generations.

Table-2. Biometric parameters of Progeny adults of Nesolynx thymus following exposure of their parent females to 32ºC in different generation

Generation (No.)	Body length(mm)		Head width(mm)		Wingspan(mm)		Female abdomen size (mm)	
	Male	Female	Male	Female	Male	Female	length	width
1	1.572± 0.017 d	2.064± 0.018 b	0.420± 0.013 d	0.570± 0.010 b	2.616± 0.024 d	3.300± 0.020 cd	0.924± 0.018 cd	0.642± 0.012 d
2	1.548± 0.015 d	2.112± 0.028 b	0.384± 0.010 e	0.570± 0.010 b	2.532± 0.022 e	3.312± 0.028 cd	0.948± 0.027 bcd	0.660± 0.015 cd
3	1.560± 0.020 d	2.094± 0.021 b	0.402± 0.013 de	0.576± 0.098 b	2.628± 0.040 d	3.348± 0.022 abc	0.954± 0.021 bcd	0.654± 0.014 d
4	1.566± 0.014 cd	2.118± 0.018 b	0.480± 0.009 bc	0.606± 0.011 bc	2.994± 0.019 c	3.354± 0.024 abc	0.984± 0.018 abc	0.714± 0.017 ab
5	1.614± 0.014 c	2.064± 0.010 b	0.474± 0.014 bc	0.588± 0.015 b	3.000± 0.018 c	3.276± 0.013 d	0.984± 0.010 abc	0.648± 0.015 d
6	1.800± 0.013 ab	2.112± 0.012 b	0.540± 0.013 a	0.648± 0.012 a	3.096± 0.026 b	3.366± 0.016 abc	1.008± 0.012 ab	0.732± 0.012 a
7	1.758± 0.020 b	2.118± 0.018 b	0.504± 0.018 b	0.630± 0.013 ab	3.162± 0.022 ab	3.324± 0.016 bcd	1.026± 0.017 a	0.702± 0.013 abc
8	1.848± 0.015 a	2.117± 0.028 a	0.552± 0.008 a	0.654± 0.019 a	3.204± 0.013 a	3.342± 0.020 abcd	1.002± 0.024 ab	0.684± 0.010 bcd
9	1.846± 0.017 a	2.214± 0.019 a	0.558± 0.009 a	0.660± 0.015 a	3.198± 0.016 a	3.408± 0.017 a	1.002± 0.020 ab	0.732± 0.012 a
10	1.836± 0.016 a	2.196± 0.024 a	0.552± 0.008 a	0.654± 0.017 a	3.126± 0.017 b	3.390± 0.029 ab	1.002± 0.022 ab	0.714± 0.017 ab
Control	1.770± 0.010 b	2.106± 0.014 b	0.488± 0.008 c	0.606± 0.014 bc	3.150± 0.013 ab	3.318± 0.020 cd	0.912± 0.017 d	0.666± 0.014 cd
F value	65.560 **	6.277 **	29.555 **	6.966 **	134.851 **	3.442 **	3.700 **	5.988 **

Values given in the Table are the mean of 5 replications (Mean ± SE);
Mean values followed by the same superscript in columns are statistically not significant;
@ - Out of 5 pupae provided for parasitization;
** - Significant at 1 %;

Wingspan

Male:

The male wingspan was significantly more (P≤0.01) for the parasitoid males emerging in generations 8 (3.204±0.016 mm) and 9 (3.198±0.016 mm) compared to that for the adults recovered from the remaining treatments.

Female:

The mean values for female wingspan fluctuated between 3.276±0.013 mm (generation-5) and 3.408±0.017 mm (generation 9) among the treatments. The female wingspan was significantly greater (P≤0.01) at generation 9 compared to the remaining treatments.

Female Abdomen Size:

Length:

Significantly higher (P≤0.01) female abdomen length was recorded at generation 7 (1.026±0.017 mm). The mean values ranged from 0.924±0.018 mm (generation 1) to 1.026±0.017 (generation 7) among the treatments.

Width:

The female abdomen width varied from 0.642±0.012 (generation 1) to 0.732±0.012 (generation 6 and 9) where it was significantly higher (P≤0.01) than the remaining treatments.

DISCUSSION

Temperature is one of the critical factors in the development of insects (Miller, 1992, 1996; Gullan and Cranston, 1994; Zeiss *et al.,* 1996). Development is prolonged and mortality increased at a cool or hot extremes. (Ables *et al.,* 1976; Madar and Miller, 1984; Cheah, 1987; Cave and Gaylor, 1988; Miller, 1996). Individual species and the different stages in their life history may develop at specific rate in relation to temperature and physiological time, a measure of the amount of heat required over time for an insect to complete development is meaningful for biological control (Miller, 1996; Gullan and Cranston, 1994; Aroga and Coderre, 2001). Oviposition and longevity are two parameters influenced by temperature (Chapman, 1969).

Longevity is generally inversely related to temperature while a highest oviposition rate is found around the median values of the range of temperature where the development occurs (Chapman, 1969).

Effect of constant temperature on the developmental characters of egg, larval and pupal parasitoids have been well documented (Kishore *et al.,* 1994; Ruberson *et al.,* 1995; Miller, 1996; Omer *et al.,* 1996; Olaye *et al.,* 1997; Aroga and Coderre, 2001; Urbaneja *et al.,* 2002; Bazzocchii *et al.,* 2003; Grassberger and Frank, 2003; Ichiki *et al.,* 2003; Maceda *et al.,* 2003; Murthy *et al.,* 2003; Daane *et al.,* 2004; Ohta and Ohtaishi, 2004; Pratissoli *et al.,* 2004; Islam *et al.,* 2005; Leopold *et al.,* 2005; Chong and Oetting, 2006). The developmental parameters, which have been chiefly studied, include developmental duration, parasitization rate progeny production and sex ratio. A few authors have made an attempt to determine the maximum and minimum threshold temperatures as well as optimum temperature required for development of parasitoids (Obrycki *et al.,* 1987; Ruberson *et al.,* 1995; Olaye *et al.,* 1997; Bazzocchi *et al.,* 2003, Grassberger and Frank, 2003; Ichiki *et al.,* 2003; Maceda *et al.,* 2003; Leopold *et al.,* 2005; Chong and Oetting, 2006).

In the present study, the parasitization potential of *N. thymus* has been found to decrease with increase in duration of exposure to natural force of physical factors like temperature and relative humidity, while the duration of exposure did not significantly affect the developmental duration.

The performance of F1 generation from parasitoid adults developed from host pupa exposed from the day of oviposition till emergence had affected the developmental duration and progeny production. The reason for declining to heat stress exerted when exposed for longer durations resulting in mortality and/or arrest of development as it is an established fact that temperatures ranging from 15 to 30ºC are fairly congenial for insect development. Further, attempts also have been made in the present investigation to evolve temperature tolerant line (population) of *N. thymus* by subjecting it to breed at a relatively higher temperature of 32ºC where 2 day-old parasitization host pupae were exposed to the chosen temperature repeatedly for 10 generations. As such, the parasitoid was able to develop successfully at this temperature. The results thus generated clearly suggest that the parasitoid has developed temperature tolerance compared to that in 10 generation before. The evolved line is expected to survive and perform parasitize relatively better against *E. bombycis* in regions where prevalence of ambient temperature would be in the range of 30ºC.

From the foraging account, it becomes evidenced that, *N. thymus* exhibits a great deal of resistance to reasonably wider range of temperature as evidenced by successful development at 15 -32 ºC.

It was observed that the parasitoid could successfully complete its life cycle at 32°C. with no significant decline in rate of parasitization upto 8th generation. Though there was no consistency in the progeny production at different generation, some decline was observed from 8th generation onwards. Further, the parasitoid sex ratio remained unchanged.

The results thus generated clearly suggest that the parasitoid has developed temperature tolerance compared to that in 10 generation before. The evolved line is expected to survive and perform relatively better against *E. bombycis* in regions where prevalence of ambient temperature would be in the neighborhood of 30ºC.

Conflict of Interests

Authors declare that there is no conflict of interests regarding the publication of this paper.

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