

## REARING OF NATURAL ENEMIES OF *PIERIS BRASSICAE* L. UNDER LABORATORY CONDITION

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### خلاصہ

ضرر رساں کیڑوں کے تدارک کے لئے ہمیں دوست کیڑوں کے بارے میں معلومات ہونی چاہئیں۔ پاکستان میں گو بھی کی سٹری کے دوست کیڑوں میں *Cotesia glomerata*, *Diadegma pierisae*, *Brachymeria lasus* اور *Pteromalus puparium* شامل ہیں۔ ان دوست کیڑوں کی دور حیات معلوم کرنے کے لئے ایگریکلچرل ریسرچ انسٹیٹیوٹ ترناب پشاور کے انٹومالوجی سکشن میں تجربات کئے گئے۔ لیڈاٹری میں تحقیق کی گئی جس میں گو بھی کی سٹری کے مختلف ادوار (Stage) کو استعمال کیا گیا۔ تاکہ دوست کیڑوں کے لئے مناسب گو بھی کی سٹری کا ادوار (Stage) معلوم کیا جاسکے۔ تجربات کے نتیجے سے ظاہر ہوتا ہے کہ *Pteromalus puparium* 22.22% *Diadegma pierisae*, *Brachymeria lasus* اور *Pteromalus puparium* کا حملہ گو بھی کی سٹری کے پانچویں دور پر ہوتا ہے۔ لیکن اس میں دوست کیڑوں کی 100% فی صد ہلاکت ریکارڈ کی گئی ہے۔ جبکہ مندرجہ ذیل کیڑوں *Diadegma pierisae*, *Brachymeria lasus* اور *Cotesia glomerata* کو جو بھی میسر گو بھی کی سٹری کا دور دیا گیا۔ اس پر دوست کیڑے کی نسل نہیں چل سکی۔

### Abstract

Knowledge of the biological attributes of parasitoids plays a fundamental role in improved management of their pest host. *Cotesia glomerata* L., *Diadegma pierisae* (Rao), *Pteromalus puparium* L and *Brachymeria lasus* Walker are major indigenous parasitoid of *Pieris brassicae* L. in different parts of Pakistan. Rearing experiments of these parasitoids was done in Entomology section Agric Res. Institute Peshawar. Set of laboratory experiments was conducted to determine the *Pieris brassicae* L. larval stage preference of *Cotesia glomerata* L., *Diadegma pierisae* (Rao), *Pteromalus puparium* L. and *Brachymeria lasus* Walker and their influence on life history traits. Results showed that maximum parasitization of *Pteromalus puparium* L. was 22.22% was in the 5<sup>th</sup> instar larva, parasitoid mortality was 100% on 5<sup>th</sup> instar and pre pupa. But no parasitization was recovered of *Diadegma pierisae* (Rao), *Cotesia glomerata* L. and *Brachymeria lasus* Walker when different instars of *Pieris brassicae* L. were provided.

### Introduction

Cabbage butterfly (*Pieris brassicae* L.) is one of the serious pest of cauliflower in many parts of Pakistan. There is number of natural enemies of this butterfly recorded in literature including *Cotesia glomerata* L., *Diadegma pierisae* (Rao), *Pteromalus puparium* L. and *Brachymeria lasus*. All these are larval and pupal parasitoids. But no natural enemy was recorded on *P. brassicae* eggs in Pakistan. (Mushtaque and Mohyuddin, 1984). But these larval and pupal parasitoids are not synchronized with its host *P. brassicae* in fruiting period of cauliflower in Peshawar valley. However, for this study cauliflower crop was kept in field after harvesting the curd just to collect natural enemies from field and to rear them in laboratory and study their biology.

### Materials and Methods

The research studies on rearing of natural enemies of cabbage butterfly, *Pieris brassicae* L. (Lepidoptera: Pieridae) on cauliflower, *Brassica oleracea* L. was conducted at the Agricultural Research Institute Tarnab, Peshawar. The natural enemies were reared on *P. brassicae* in a set of experiments. In case of larval parasitoids 4<sup>th</sup> and 5<sup>th</sup> instar larvae of *P. brassicae* were collected from field at weekly intervals for collection of *Diadegma pierisae* (Rao) and *Cotesia glomerata* L. The pre-pupa and pupa of *P. brassicae* were collected from field when it was available for *Pteromalus puparium* L. and *Brachymeria lasus* Walker emergence. The collected larvae and pupae were kept in glass chimneys under laboratory conditions of 18±2°C, 50±5% RH and 12:12h photoperiod till emergence of natural enemies. In case of parasitoids rearing couple of parasitoids adults were exposed to different instars of the host for egg laying. The parasitized hosts were observed for parasitoid

emergence. Fresh uniform sized cauliflower leaves were daily provided to the host as food in glass chimneys. Parasitoids were also provided with 10% honey solution and fresh flower of brassica as pollen source. Each set of experiment was replicated 4 times. Percent parasitism and percent parasitoid mortality was calculated by following formulae:

$$\% \text{ Parasitism} = \frac{\text{No. of parasitized host}}{\text{Total No. of hosts provided}} \times 100$$

$$\% \text{ Parasitoid Mortality} = \frac{\text{No. of parasitoid failed to emerge}}{\text{Total No. of parasitoid pupa}} \times 100$$

## Results

Table 1 showed that maximum 22.22% percent infestation was recorded when *Peteromalus puparium* was exposed to 5<sup>th</sup> instar larvae of *P. brassicae* and 12.5% peritization occur when exposed to pre pupa of *P. brassicae* but 100 % parasitoid mortality was recorded inside host pupa. No parasitization was recorded when 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> instar larvae and pupa of *P. brassicae* was exposed to *Peteromalus puparium*

Table 2 showed that when different instars of *P. brassicae* was exposed to larval parasitoid *D. pierisae* (Rao) in laboratory no parasitization was recorded and only 2 *P. brassicae* larvae complete their lifecycle and 2 male butterfly emerged.

Table 3 showed that after 24 hours parasitization by *D. pierisae* to 1<sup>st</sup> and 3<sup>rd</sup> instar of *P. brassicae* there was no parasitization observed and no *P. brassicae* larvae could complete its lifecycle.

Table 4 showed that after 4 hours parasitization by *D. pierisae* to 1<sup>st</sup> instar of *P. brassicae* there was no parasitization observed and no *P. brassicae* larvae could complete its lifecycle.

Table 5 revealed that after exposing 1st instar larvae of *P. brassicae* to *C. glomarata* no parasitization was observed and no butterfly was emerged

Table 6 showed that after parasitization of 1<sup>st</sup> and 5<sup>th</sup> instar *P. brassicae* larvae by *B. lasus* the values for average number of host pupa formed, and similar number of butterfly emerged, female to male sex ratio and percent parasitization were all nil.

## Discussion

The parasitoids emerged from *P. brassicae* larvae and pupae collected from field, were provided with different host instars to study their biology under laboratory conditions. Mean number of *Pteromalus puparium* L. host pupa formed was a maximum no. of 8 on pre-pupa and pupa and nil on 1st instar. Mean no. of adults emerged were nil from 1<sup>st</sup>, 2<sup>nd</sup> and 5<sup>th</sup> instar and maximum of 6 from pupa. *P. puparum* is a idiobiont endoparasitoid that attacks gregariously to the pupae of several butterfly families, including Pieridae (Harvey *et al.*, 2011). Female to male sex ratio was maximum of 1:3 from pre-pupa and minimum of nil from 1<sup>st</sup>, 2<sup>nd</sup> and 5<sup>th</sup> instar. Parasitization was maximum of 22.22% from 5<sup>th</sup> instar and nil from 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> instar and pupa. Parasitoid mortality was 100% from 5<sup>th</sup> instar and pre-pupa and nil from 1-4<sup>th</sup> instar and host pupa. It may be due to unsuitable host quality or environmental conditions. Mackauer and Sequeira (1993) reported that for idiobiont parasitoids, the host quality in term of its nutritional history prior to parasitism is very important. *P. puparum* can easily assess quantitative differences of their hosts and can adjust their clutch sizes accordingly (Schmidt and Smith, 1985; Harvey *et al.*, 2011). *P. puparum* determine their host size through chemical or physical changes in their host hemolymph, and regulate their number of eggs laying. (Takagi, 1986).

*P. puparum* can lays 150 eggs per gram of host (Takagi, 1986). After hatching, *P. puparum* larvae feed on the internal tissue of its host and pupate between the internal membrane and the cuticle of the host pupa. After few days adult *P. puparum* emerge (Harvey *et al.*, 2011).

In present study *Diadegma pierisae* (Rao) and *Brachymeria lasus* Walker were released on different available *P. brassicae* larval instars but no parasitization was observed. It might be due to unsuitable host instars or environmental conditions. Paul and Tumlinson (1999) reported that volatile terpenoids and other compounds emitted from leaves in response to insect damage allow insect parasitoids (such as parasitic wasps) and predators to distinguish between infested and noninfested plants, and thus aid in locating hosts or prey.

After parasitization by *C. glomarata* mean no. of *P. brassicae* host pupa formed, mean no. of adults emerged, female to male sex ratio and percent parasitization were nil from both the host stages. After parasitization by *Brachymeria lasus* Walker the values for mean no. of *P. brassicae* host pupa formed, mean no of butterfly emerged, female to male sex ratio and percent parasitization were all nil for both host stages.

Among larval parasitoid of *P. brassicae*, *C. glomerata* was most successful it can cause 100% parasitization. *C. glomerata* is a koinobiont endoparasitoid that parasitizes gregariously to L1-L3 larval instar

of *P. brassicae* and related species in the Pieridae (Feltwell, 1982). *C. glomerata* wasp lays 20–30 eggs in a single first instar larva of *P. brassicae*. Parasitoid larvae are present in different parts of the host's body. Late instar host larvae contain full grown second and third instar *C. glomerata* larvae (Gu *et al.*, 2003; Masurier, 1991). In present study *C. glomerata* was released on newly hatched and 2 days old 1<sup>st</sup> instar *P. brassicae* larvae up till it entered in next instar for egg laying but host larvae could not complete its lifecycle and died in 2<sup>nd</sup> and 3<sup>rd</sup> instar, and thus no parasitization was observed. The failure of parasitization might be due to unsuitable host plants as these were late season varieties, which were usually not grown in this area, or due to unsuitable environmental conditions. Taiadjana *et al.* (2012) reported that the survival of *C. glomerata* differed according to the plant species where its host larva had fed on. The wasp has 49% survival on that hosts which developed on the native plant while 1% of the wasps survived on the exotic plant. They suggested that the diet of larval host has a negative effect on the *C. glomerata* development, its immature mortality and small adult size.

**Table 1. Rearing of *P. puparium* on *P. brassicae* larvae.**

Host stage	Mean No. of larvae used	Pair of parasitoid released	Mean No. of host pupa formed	Mean No. Butterfly emerged	Sex ratio of butterfly Female : Male	% parasitization	Parasitoid emerged	Parasitoid % Mortality
1 <sup>st</sup> instar	18	5	0	0	0	0	0	0
2 <sup>nd</sup> instar	13	5	1.6	0	0	0	0	0
3 <sup>rd</sup> instar	10	4	6.5	5.5	1.2:1	0	0	0
4 <sup>th</sup> instar	7	3	2.5	1.5	1:1	0	0	0
5 <sup>th</sup> instar	5	3	3.66	0	0	22.22	0	100
Pre pupa	8	3	8	4	1:3	12.5	0	100
Pupa	8	3	8	6	1:1	0	0	0

**Table 2. Rearing of *D. pierisae* on *P. brassicae* larvae. (Provided parasitoid with host larvae for stadium)**

Host stage	Mean No. of larvae used	Pairs of parasitoid released	Mean No. of host pupa formed	Mean No. Butterfly emerged	Female : Male Sex ratio	% parasitization
1 <sup>st</sup> instar	18	1	0.3	0	0	0
2 <sup>nd</sup> instar	26	1	3	2	0 : 2	0
3 <sup>rd</sup> instar	17	1	0.25	0	0	0
4 <sup>th</sup> instar	7	1	0.33	0	0	0

**Table 3. Rearing of *D. pierisae* on *P. brassicae* larvae. (Provided parasitoid with host larvae for 24h only)**

Host stage	Mean No. of larvae used	Pair of parasitoid released	Mean No. of host pupa formed	Mean No. Butterfly emerged	Female: Male Sex ratio	% parasitization
1 <sup>st</sup> instar	18	1	0	0	0	0
3 <sup>rd</sup> instar	10	1	2	0	0	0

**Table 4. Rearing of *D. pierisae* on *P. brassicae* larvae. (Provided parasitoid with host larvae for 4h only)**

Host stage	Mean No. of larvae used	Pair of parasitoid released	Mean No. of host pupa formed	Mean No. Butterfly emerged	Female : Male Sex ratio	% parasitization
1 <sup>st</sup> instar	15	1	2	0	0	0

**Table 5. Rearing of *Cotesia glomarata* L. on *P. brassicae* larvae. (Provided parasitoid for stadium)**

Host stage	Mean No. of larvae used	Pair of parasitoid released	Mean No. of host pupa formed	Mean No. Butterfly emerged	Female : Male Sex ratio	% parasitization
1 <sup>st</sup> instar	18	4	0	0	0	0
1 <sup>st</sup> instar (2 days old)	27	3	0	0	0	0

**Table 6. Rearing of *B. lasus* on *P. brassicae* larvae. (Provided parasitoid for stadium)**

Host stage	Mean No. of larvae used	Pair of parasitoid released	Mean No. of host pupa formed	Mean No. Butterfly emerged	Female : Male Sex ratio	% parasitization
1 <sup>st</sup> instar	12	1	0	0	0	0
5 <sup>th</sup> instar	14	1	0	0	0	0

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### References

- Feltwell, J. (1982). *Large White Butterfly: The biology, biochemistry and physiology of Pieris brassicae* (L.). Junk Publishers, Hague, Netherlands.
- Gu, H., Wang, Q. and Dorn, S.(2003). Superparasitism in *Cotesia glomerata*: response of hosts and consequences for parasitoids. *Ecol. Entomol.* 28: 422-431.
- Harvey, J.A., Van Dam, N. M., Raaijmakers, C. E., Bullock, J. M. and Gols, R. ( 2011). Tritrophic effects of inter- and intra-population variation in defence chemistry of wild cabbage (*Brassica oleracea*). *Oecologia*, 166: 421- 431.
- Mackauer, M. and Sequeira, R. (1993). Patterns of development in insect parasites. In: Beckage, N.E., S.N. Thompson and B.A. Federici (Eds.). *Parasites and Pathogens of Insects*. Academic Press, New York, NY, USA, pp. 1–20.
- Masurier, Le. A. D. (1991). Effect of host size on clutch size in *Cotesia glomerata*. *J. Anim. Ecol.* 60: 107–118.
- Mushtaque, M. and Mohyuddin, A.I. (1984). *Pieris brassicae* (Pieridae: Lepidoptera), A pest of crucifers and its control by parasites. *Pak. J. Agric.Res.* 5(3): 165-169
- Paul, W. P. and Tumlinson, J. H. (1999). Plant volatiles as a defense against insect herbivores. *Amer. Soc. Pl. Physiologists.* 121: 325-331
- Schmidt, J.M. and. Smith, J.J.B. (1985). Host volume measurement by the parasitoid wasp *Trichogramma minutum*: the roles of curvature and surface area. *Entomol. Exp. et Appl.* 39: 213-221.
- Taiadjana, M.F., Louise, E.M.V. and. Harvey, J.A. (2012). Effects of an invasive plant on the performance of two parasitoids with different host exploitation strategies. *Biol. Contr.* 62: 213–220.
- Takagi, M. (1986). The reproductive strategy of the gregarious parasitoid, *Pteromalus puparum* (Hymenoptera, Pteromalidae) to host size discrimination and regulation of the number and sex ratio of progeny in a single host. *Oecologia*, 70: 321- 325.