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Effect on total protein concentration (mg/g) of host larvae due to the frequent parasitization and time after parasitization by *Bracon hebetor* (Say) (Hymenoptera: Braconidae)

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Abstract
This experiment was conducted to find out the impact of frequency of parasitization and time after parasitization on the host protein level parasitizing by *Bracon hebetor* (Say) which can be helpful in mass rearing of this parasitoid. This species acts as a biological control agent of the Indian meal moth *Plodia interpunctella* (Hübner) which is a serious pest of stored grain food products. The result of this work showed that there is significant difference among the frequency of parasitization by *B. hebetor* on the protein levels of the host larvae (F= 56.92, $P<0.001$) and the role of the physiological effects of the venom of *B. hebetor* on the total protein concentration of the larvae of *P. interpunctella* was not significant ($P>0.05$) but has mild effect due to the increase of the days after parasitization.

Keywords: Indian meal moth, parasitoid, protein concentration, frequency of parasitization

Introduction
The application of chemical pesticides should be decreased as it has some prominent drawbacks and for that implementation of biological control method has been encouraged day by day (Bale *et al*. 2008) [2]. Plant derived antagonists, toxins of insect (Whetstone and Hammock, 2007) [14] and natural ecofriendly pesticides (Dayan *et al*. 2009) [7] are also being considered. The parasitoid wasp *Bracon hebetor* Say is an effective biological control agent used against some stored product lepidopteran pests (Darwish *et al*. 2003) [6]. For some positive features like high reproductive rate, short life span and wide range of host species it has been considered in various experiments regarding parasitoid-host relationship (Yu *et al*. 2003) [15].

For mass production of bio-control agents biochemical and physiochemical studies of parasitoid-host relationship is essential (Nakamatsu and Tanaka, 2003) [9]. The infestation of this parasitoid initiates some physiological changes like stupor, braking development and immune system of the host (Moreau and Guillot, 2005) [8].The infestation of *Bracon hebetor* also affects the biogenesis and different host factors (Quistad *et al*. 1994) [10].

Different quantitative and/or qualitative changes occur due to the envenomation of parasitoid in the host plasma protein level (Rahbe *et al*. 2002) [11] which may be governed in the host body for the growth of parasitoid (Beckage and Kanost, 1993) [3]. The toxic proteins injected by *Bracon hebetor* may affect the intersynaptic conduction in the host larvae since it hampers the exocytosis of presynaptic vesicles (Walther and Reinecke, 1983) [13]. The females of this parasitoid prefers to infest the 5th (last) instar host larvae (Benson, 1973) [4] and the infested larvae rapidly and instantly paralyzed (Shim *et al.* 2008) [12]. In this research work it is studied whether frequency of parasitization and time after parasitization by *B. hebetor* has effect on the concentration of total protein level of the host Indian meal moth (IMM).

Materials and Methods
Materials
1. Host larvae: Mature (5th instar) larvae of *P. interpunctella* was taken as host from the rearing culture jar (size - height =25 cm and diameter =11cm) on artificial diets of maize flour. The 5th instar larvae of *P. interpunctella* were obtained from the Post Harvest Laboratory, Department of Zoology in University of Rajshahi.

2. Parasitoid *B. hebetor*: The parasitoid *B. hebetor* was taken from a stock culture of *B. hebetor* reared on fifth instar larvae of P. *interpunctella*. The *B. hebetor* was also obtained from the Post-Harvest Laboratory, Department of Zoology in University of Rajshahi.

Methods

Effect of frequency of Parasitization

6 containers were prepared by washing and cleaning and sterilized in sunlight. Then 6 host larvae were released with the help of insect forceps into each of the containers and 1pair of *B. hebetor* was also released into each of the containers and allowed to parasitize the hosts for about 12 hours. After Parasitization the paralyzed larvae were shifted into the sterilized petridishes (After 1st frequency of Parasitization) and analyzed the protein concentration and then another group of host larvae ware released into the previous container of same *B. hebetor*. After 12 hours the paralyzed larvae were taken off for analyzing the protein concentration. This process was continued up to 6th frequency of Parasitization.

Effect of time after Parasitization

5 containers were prepared by washing and cleaning and sterilized in sunlight. Then 10 host larvae were given with the help of insect forceps into each of the containers and 1pair of *B. hebetor* was also released into each of the containers and allowed to parasitize the hosts. After Parasitization the paralyzed larvae were shifted into the sterilized petridishes. The one day old parasitized larvae were subjected to protein analysis.

Next day, the two days old parasitized larvae were subjected to protein analysis. Thus the third, fourth and fifth days old parasitized larvae were used for protein analysis.

Protein analysis: For analyzing the protein concentration the following steps were followed.

i. Protein precipitation

1. At first a solution of 4M $(NH_4)_2SO_4$ was prepared in a test tube. Then 0.1M Phosphate buffered saline (PBS) solution was prepared in a glass jar.
2. Crushing of parasitized larvae: The larvae were crushed and grinded by using mortar and pestle. The grinded parts were shifted into an eppendorf tube and 1 ml PBS were added. Then it was vortexed and centrifuged at the speed of 1350 rpm for 10 minutes. Then the supernatant was separated into another tube and $(NH_4)_2SO_4$ was added instillingly and vortexed. Then the precipitated parts were separated into a new eppendorf tube and it was kept in ice incubator for 5 minutes and again centrifugation was done for 5 minutes and finally the precipitated protein was found.

ii. Measuring protein concentration

The protein concentrations were calculated according to the Bradford Process (Bradford, 1976) [5], using Coomassie brilliant blue G250 (CBB) protein-assay reagent at 595 nm (Eppendorf Bio Photometer). A standard curve was prepared using bovine serum albumin as the standard. Each sample was analyzed in three time and the experiment was repeated thrice. In the next step, a series of protein standards diluted with 0.15 M NaCl to final concretion of 0 (blank distilled water only), 25, 50, 75, 100 mg/ml and also a serial dilutions of the sample was prepared. Then 100 µl of each of the above sample kept to separate tubes and 1 ml of CBB was added to each of the tubes. Then it was subjected to vortex for 5 minutes and after 5 minutes spectrophotometer was turned on and adjusted to a wavelength of 595 nm. It was waited for 5 minutes and the reading of the absorbance was recorded.

Finally, the absorbance of the standards vs. their concentrations were plotted in graph using Microsoft excel software. The concentration of total protein was calculated by using the Calibration curve.

Results

Effect of frequency of Parasitization

In case of 25 µl solution, the maximum concentration of total protein was found in 1st frequency of Parasitization (26.77±0.03 mg/g) and minimum in 6th frequency of Parasitization (14.81±0.01 mg/g) (Table 1). In case of 50 µl solution the maximum concentration of total protein was found in 1st frequency of Parasitization (35.54±0.05 mg/g) and minimum in 6th frequency of Parasitization (18.69±0.03 mg/g) (Table 1). In case of 75 µl solution the maximum concentration of total protein was found in 1st frequency of Parasitization (38.01±0.11 mg/g) and minimum in 6th frequency of Parasitization (21.78±0.04 mg/g) (Table 1). In case of 100 µl solution the maximum concentration of total protein was found in 1st frequency of Parasitization (26.77±0.03 mg/g) and minimum in 6th frequency of Parasitization (24.99±0.01 mg/g) (Table 1).

The present result showed that there is significant difference among the frequency of Parasitization by *B. hebetor* on the protein levels of IMM (F= 56.92, F Crit= 2.90).

Table 1: Effect on total protein concentration (mg/g) of host larvae due to the frequent Parasitization by *B. hebetor*

Frequency of Parasitization	Protein concentration (mg/g) Mean ± SE			
	25µl	50 µl	75 µl	100 µl
1st	26.77±0.03	35.54±0.05	38.01±0.11	40.10±0.04
2nd	22.73±0.05	33.49±0.04	36.51±0.01	38.92±0.12
3rd	17.81±0.01	24.17±0.04	29.67±0	33±0.06
4th	17.44±01	20.87±0.02	22.74±01	24.93±0.02
5th	15.44±0.02	20.13±0.03	24.67±0.04	26.76±0.06
6th	14.81±0.01	18.69±0.03	21.78±0.04	24.99±0.01

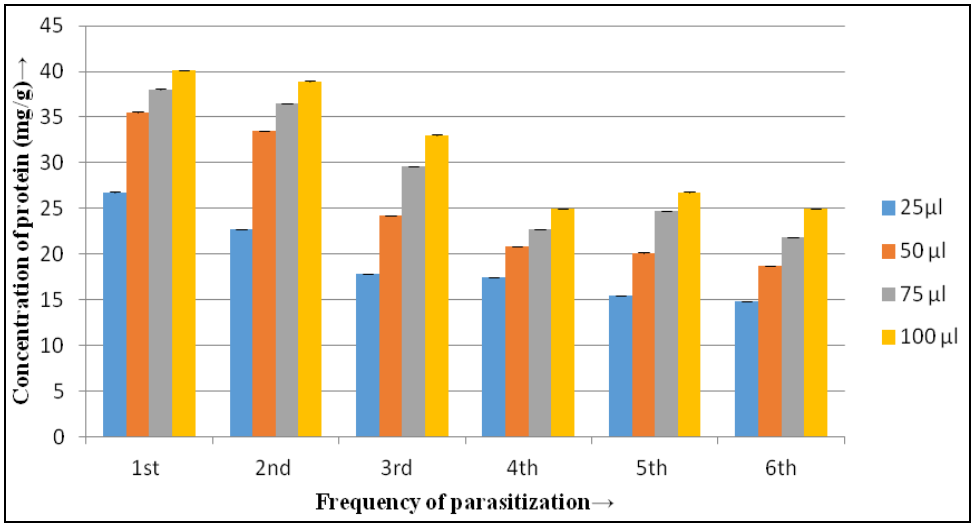

Fig 1: Effect on total protein concentration (mg/g) of host larvae due to the frequent Parasitization by *B. hebetor*.

Effect of time after Parasitization

In this research in case of 25 µl solution, the maximum concentration of total protein was found in 5th day after Parasitization (27.14±0.01 mg/g) and minimum in 1st day after Parasitization (12.26±0.04 mg/g) (Table 2). In case of 50 µl solution the maximum concentration of total protein was found in 5th day after Parasitization (30.65±0.02 mg/g) and minimum in 1st day after Parasitization (16.56±0.01mg/g) (Table 2). In case of 75 µl solution the maximum concentration of total protein was found in 5th day after Parasitization (31.72±0.02 mg/g) and minimum in 1st day after Parasitization (20.36±0.05 mg/g) (Table 2). In case of 100 µl solution the maximum concentration of total protein was found in 5th day after Parasitization (32.91±0.06 mg/g) and minimum in 1st day after Parasitization (21.76±0.04 mg/g) (Table 2).

Table 2: Effect on total protein concentration (mg/g) due to the time after Parasitization

Samples	protein concentration (mg/g) Mean ± SE			
	25µl	**50 µl**	**75 µl**	**100 µl**
1st day after parasitization	12.26±0.04	16.56±0.01	20.36±0.05	21.76±0.04
2nd day after parasitization	17.90±0.02	20.4±0.13	22.53±0.05	23.01±0
3rd day after parasitization	20.54±0.01	26.32±0.03	27.32±0.03	28.47±0.03
4th day after parasitization	20.71±0.01	24.36±0.03	26.74±0.07	27.17±0.06
5th day after parasitization	27.14±0.01	30.65±0.02	31.72±0.02	32.91±0.06

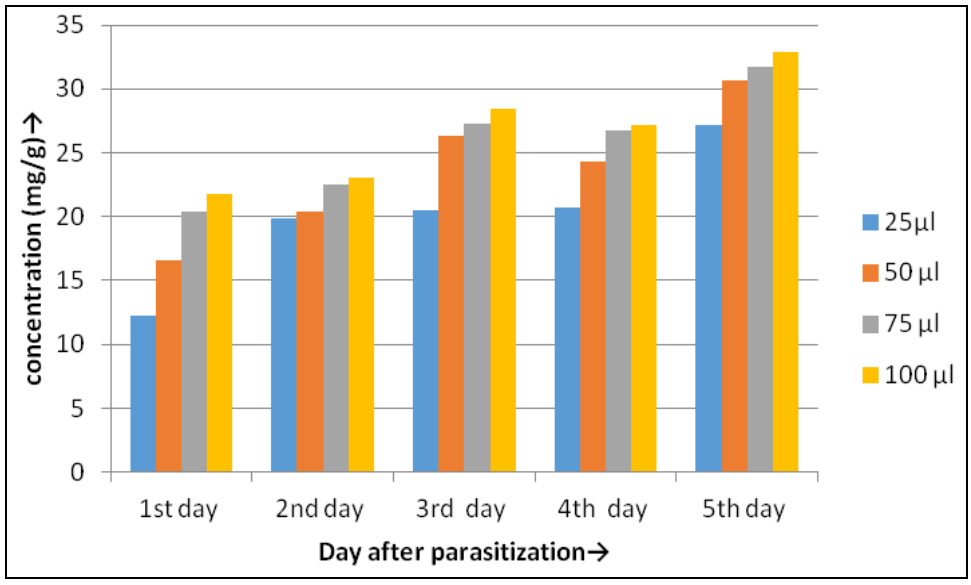

Fig 2: Effect on total protein concentration (mg/g) in different days after Parasitization

The Spectrophotometric analysis of total protein present in the larvae of *P. interpunctella* 1st day, 2nd day, 3rd day, 4th day and 5th day after Parasitization by *B. hebetor* showed that there was a slight effect on total protein concentration (mg/g) in different days after Parasitization but it was not statistically significant (P>0.05).

Discussion

A few works have been found regarding this work. This experiment supports the findings of Rahbe *et al.* (2002) [11] according to whom the infestation of parasitoid has effects on the host plasma protein. The infested host larvae became immobilized rapidly and instantly which shows similarity

with the findings of Shim *et al.* (2008) [12] though Baker and Fabrick (2000) [1] found no significant differences in host hemolymph proteins up to 3 days after parasitization by *B. hebetor*.

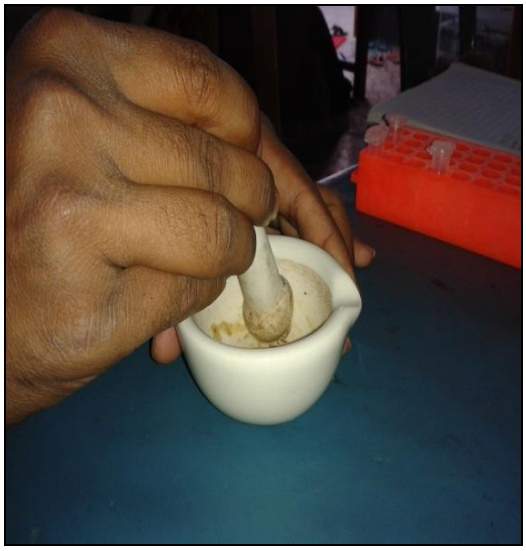

Plate 1: Crushing of host larvae

Plate 2: Ice incubation of sample

Plate 3: Samples in tray

Plate 4: Colour change after adding CBB

Plate 5: Spectro-photometer

Conclusion

It can be concluded that these results will help in mass rearing of *B. hebetor* in the laboratory condition for fruitful research and understanding the biology of this particular pest. To know more about this future studies should also be focused for the practical applications in the field level.

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