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Analysis of the Effects of Magnetic Field on the Haemogram Profile and Protein Content of Eri Silkworm, *Philosamia ricini*

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Abstract

The geomagnetic field is a naturally occurring physical force that operates to sustain life on earth. However, recent advances in technology, rapid urbanization and electromagnetic pollution have led to interferences in strength of the magnetic field, which has resulted in various health hazards in several life forms. This study was carried out to understand the effect of magnetic fields that are greater than the strength of the natural geomagnetic field in *Philosamia ricini*, commonly known as Eri silkworm. Qualitative and quantitative analysis of the haemogram profile as well as the total protein content, were considered for analysis. The 5th instar silkworm larvae were exposed to two magnetic fields of different strengths, namely 0.4493G and 0.8345G, and changes were recorded and compared with a control group. The results revealed decrease in the number of haemocytes and disruption of haemocytes that increased with increase in strength of magnetic field along. The total protein content of haemolymph also underwent changes with changes in the strength of the magnetic field. Such studies and future in-depth analysis of different biological parameters in organisms due to the changing geomagnetic field might be beneficial in understanding and maneuvering the effects of magnetic field on organisms.

Keywords: Electromagnetic Pollution, Haemogram, Magnetic Field, Silkworm

1. Introduction

The motion of electric charges creates a spherical area which is known as a magnetic field. A geomagnetic field exists on earth, which is responsible for numerous phenomena and is a prominent environmental factor influencing life on earth, directly or indirectly. Life on earth has been continuously shaped, molded and changed by evolution for more than 2 million years in the presence of a natural geomagnetic field. However, human activities have induced changes of varying degrees in this natural geomagnetic field which might be due to rigorous industrialization and additional static, affecting the natural balance in which life forms exist. Studies

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have revealed the prolonged effect of strong magnetic fields greater than that of the natural geomagnetic field in all organisms. Even in humans it can cause severe health hazards and diseases like cancer[1]. Thus, the effect of magnetism on biological system has been a topic of considerable world-wide interest for many years[2,3].

Insects are commonly used for the study of electromagnetic effects on them as they can detect and respond to magnetic fields[4]. Nowadays, the magnetic field has also been used in pest control as the exposure of biological systems to a stronger magnetic field induces behavioral[5], morphological[6], physiological and biochemical[7-10] changes in the pests. Magnetic fields have also been shown to affect the fecundity and behavior[11] of a wide variety of insects, including the silkworms, which are widely used as model organisms. Changes have been seen in the feeding behaviour of silkworm[12], silk gland proteins and mid-gut protease[13] along with glycogen contents in the body of silkworm[14].

Philosamia ricini, commonly known as Eri silkworm, is a sericogenous insect of great economic value and endemic to Assam. The present study has been designed with the aim to scrutinize the effect of the magnetic field on its haemogram profile and haemolymph protein content. Haemogram is an account of the various hemocytes, their number and types. Haemogram estimation assumes importance, and these hemocytes are accountable for various physiological processes. They are the main component of cellular immune responses and have proven their ability to mediate diverse immune defense against pathogens and other adverse conditions of stress, like temperature, starvation, photoperiod, humidity, etc[15]. Therefore, both quantitative and qualitative estimation of haemocytes are considered as an important parameter of the overall health of insects. Along with that, any kind of toxicity induced by physical or chemical alterations in and around organisms tends to easily affect the protein content or total protein of organisms. Hence, alteration in the overall protein content of organisms can be a very good indicator of changes in the environment, induced by various factors, be it toxins or otherwise.

As such, this study has been conducted to understand the changes in the haemogram of the Eri silkworm (5th instar larva) along with changes in protein content when exposed to two different magnetic fields of strength 0.4493G and 0.8345G. The fields were chosen as these field strengths are two times and three times more than the strength of the natural geomagnetic field that exists on Earth.

2. Materials and Methods

2.1 Insect

The seeds of the test insect *Philosamia ricini* were collected from "Collected Mulberry Garden" (CMG), Barpeta district of Assam and were reared in the laboratory of the Zoology Department on castor (*Ricini communis*) leaves. 5th instar larvae (4-5 days post moult) were used as these larvae possess the full complement of haemocytes[16].

2.2 Experimental Design

The magnetic field for the experiment was created by taking two circular magnets of 14 cm diameter, which have been placed parallel to each other and connecting them with a battery. For this study, two magnetic fields of different strengths have been used. The strength of these fields was 0.4493G and 0.8345G which can be gained after connecting both the magnets with batteries of two different power voltages 14mA and 26mA respectively. Larvae were exposed to the magnetic field inside a transparent container with some small holes on its surface for air circulation, and they were fed at regular intervals with castor leaves. For the exposure strength 0.4493G, 10 larvae were used, out of which 5 were examined after 24 hrs, and the rest 5 were examined after 48 hrs, duly noting the changes after each time interval. The same procedure was followed to maintain a magnetic field strength of 0.8345G for another batch of 10 silkworm larvae. A control group of 10 larvae of the same age group were examined parallelly to establish a comparison.

2.3 Quantitative Analysis of Haemocytes

To understand the effects of the chosen magnetic fields on the number of haemocytes of the 5th instar Eri silkworm larva the Total Haemocyte Count (THC) was analyzed. The larvae were removed from the container after exposure to the magnetic field and fixed in hot water at 56-60 for 2 minutes. Then they were dried on filter paper. A proleg on the 7th abdominal segment of the larvae was cut at the tip and the first few drops of haemolymph were collected. By means of a haemocytometer, haemocyte

count was carried out using the following formula suggested by Jones[17].

$$\frac{\text{Haemocytes in X mm square X dilution X depth of chamber}}{\text{No. of 1mm square counted}}$$

where, Dilution = 20 times, Depth factor of the chamber = 10 (constant) and Number of squares counted = 4

2.4 Qualitative Analysis of Haemocytes

The effect of the magnetic fields was also analyzed on the overall form, shape and structure of the haemocytes of the silkworm larvae. For that, one drop of haemolymph was drawn on a glass slide which was already washed with 100% alcohol-soaked cotton to remove any impurities. A smear was prepared, air dried for 2-3 mins, fixed and stained with Giemsa. Excess stain was removed and microscopic observation was carried out for differential cell count at 40X magnification.

2.5 Protein Estimation

For haemolymph protein estimation, the total protein was determined according to the method specified by Lowry *et al.*,[18] and OD was measured by using a spectrophotometer at 680nm.

2.6 Data Analysis

Statistical analysis was performed using MS Office 2017 and SPSS 21. Means of THC and protein content were compared by Student's t-test to find out if there was any significant difference between the control and treated group. The results were expressed as mean ± standard deviation (X ± S.D).

3. Results

3.1 Total Haemocyte Count (THC)

3.1.1 THC of the 5th Instar (4 Day Post Moult) P. ricini under Magnetic Field of 0.4493G for 3 hrs

Result of the effect of a magnetic field of strength 0.4493G for 3 hrs on 5th instar (4-day post molt) showed the changes in THC post exposure. In comparison to the control group having THC 8066.33182.94/mm^3, the treated groups of intervals 24 hrs post exposure and 48 hrs post exposure both recorded significantly lower THC, i.e., 6175452.07/mm^3 and 5786846.81/mm^3 respectively. This indicates a significant effect of the said magnetic field on haemocyte count when the larvae were exposed continuously for 3 hrs. However, non-significant difference between the exposed groups suggest that the effect of magnetic field is instantaneous and not prolonged.

3.1.2 THC of the 5th Instar Larvae on 4th Day Post Moult P. ricini under Magnetic Field of 0.4493G for 6 hrs

The THC values of larvae exposed to the same magnetic field 0.4493G but for a longer duration, i.e., 6 hrs studied after 24 hrs and 48 hrs post-exposure, recorded significantly lower numbers of haemocytes, i.e., 5191.83345.40/mm^3 and 4981.5218.75/mm^3 respectively in comparison to the control group. Here also, no prolonged effect is suggested as there are no significant differences in THC between the two treated groups.

3.1.3 THC of Larvae of P. ricini under the Magnetic Field 0.8345G for 3 Hrs

When the magnetic field strength was increased to 0.8345G, more or less similar observations were obtained. There were significant differences in THC of exposed groups in comparison to the control group (4 days post molt). The 24 hrs and 48 hrs exposed groups exhibited significantly lower counts of THC 4428.5626.08/mm^3 and 4443.83626.08/mm^3 respectively, suggesting a significant effect of the magnetic field on THC.

3.1.4. THC of 5th Instar Larvae under the Magnetic Field 0.8345 for 6 hrs

The THC values of the exposed larvae to the same magnetic field 0.8345G for a duration of 6 hrs, after 24 hrs and 48 hrs post-exposure recorded a significant decrease in numbers of haemocytes i.e., 4388.5/mm^3 and 4221.5/mm^3 respectively in comparison to the control. Hence, no prolonged effect is suggested as there is no significant difference in THC between the two exposed groups.

An interesting observation was that when the THC of the treated groups under magnetic strength 0.4493G and 0.8345G was compared (Table 1), they were found to be significantly different from each other. It suggested that the strength of the magnetic field greater is the effect on circulating haemocytes. However, the THC of silkworm

Table 1. Comparison of THC of exposed groups of larvae under magnetic field strength 0.4493G and 0.8345 G for different time intervals

Magnetic field	Control group	0.4493 G		0.8346 G	
		Observation after 24 hrs post exposure	Observation after 48 hrs post exposure	Observation after 24 hrs post exposure	Observation after 48 hrs post exposure
3 hr exposure	8066.33±182.94	6175±452.07	5786.66 ±846.8	4442.5 ±626.08	4443.83 ±597.0
6 hr exposure	8136.25±3062.4	5191.83 ±345.4	4981.5 ±218.75	4388.5 ±895.28	4221.5 ±256.29

Data are expressed as mean ± SEM. Values are significantly different at $p<0.05$. All data are in per mm^3 ($/mm^3$).

groups on exposure to the same magnetic field 24 hrs and 48 hrs was not seen to be much different from each other. The differences being non-significant suggest that the duration to exposure of the same field does not alter the effect of the magnetic field on THC and whatever effect was observed had taken place in the early stage of exposure itself.

3.2 Qualitative Analysis of Haemocytes

Study on the features of the haemocytes of the *P. ricini* (control group) 5th instar (4 days post molt) revealed five different kinds of haemocyte. Identification was carried out following Jones and Gupta[19,20]. Prohaemocyte: Pro-haemocytes were found to be small, pale grey often intensely basophilic stem cell (Yeager)[21]. Plasmatocyte: They mostly were seen as round, fusiform and spindle shaped with a small nucleus. Granulocyte: These were observed as rounded and ovoid in shape. The nucleus was small and located in the centre, surrounding which abundant cytoplasm granules are present. Spherulocyte: These cells were observed as oval in shape with a prominent but small nucleus. The cytoplasm contained spherules, and it was thick and homogenous. Oenocyte: The cells were found to be large and thick, which may be oval or elongated in shape mostly. The nucleus is often eccentrically positioned.

Study was also carried out under the same strength of magnetic fields, i.e., 0.4493G and 0.8345G. Haemolymph were collected from the same group of larvae exposed for 3 hrs and 6 hrs and studied after 24 hrs and 48 hrs. The quality of haemocytes of exposed groups was compared with the control group, which revealed drastic changes that are depicted in Figures 1 and 2. Disintegration of the haemocytes in the exposed group suggests that higher magnetic fields damage the haemocytes in insects, which holds great significance in future study of insect control.

3.3 Haemolymph Protein Content

Under the effect of magnetic field strength of 0.4493G, when larvae were exposed for 3 hrs, a gradual increase in haemolymph protein was seen in the treated group as compared to control group in 24 hrs, i.e., 36.017.64 and then a slight decrease was detected i.e., 33.22.21 in 48 hrs post exposure. When the exposure time duration was 6 hrs, a greater value of protein content was observed under the same magnetic field, i.e., 40.81.65 in 24 hrs and 40.461.16 in 48 hrs post exposure as shown in Figure 3(A).

Similar results were obtained under magnetic field 0.8845G suggesting a positive effect on haemolymph protein upon exposure. 0.8845G magnetic field was applied for 3 hrs and the larvae were studied after 24 hrs and 48 hrs where the protein content was found to be 37.56 and 35.397 respectively. Similarly, under the same magnetic field, when larvae were exposed for 6 hrs the results showed slight increase in haemolymph protein contents i.e., 35.428 in 24 hrs and 35.96 in 48 hrs as shown in Figure 3(B). However, the increase is not significant.

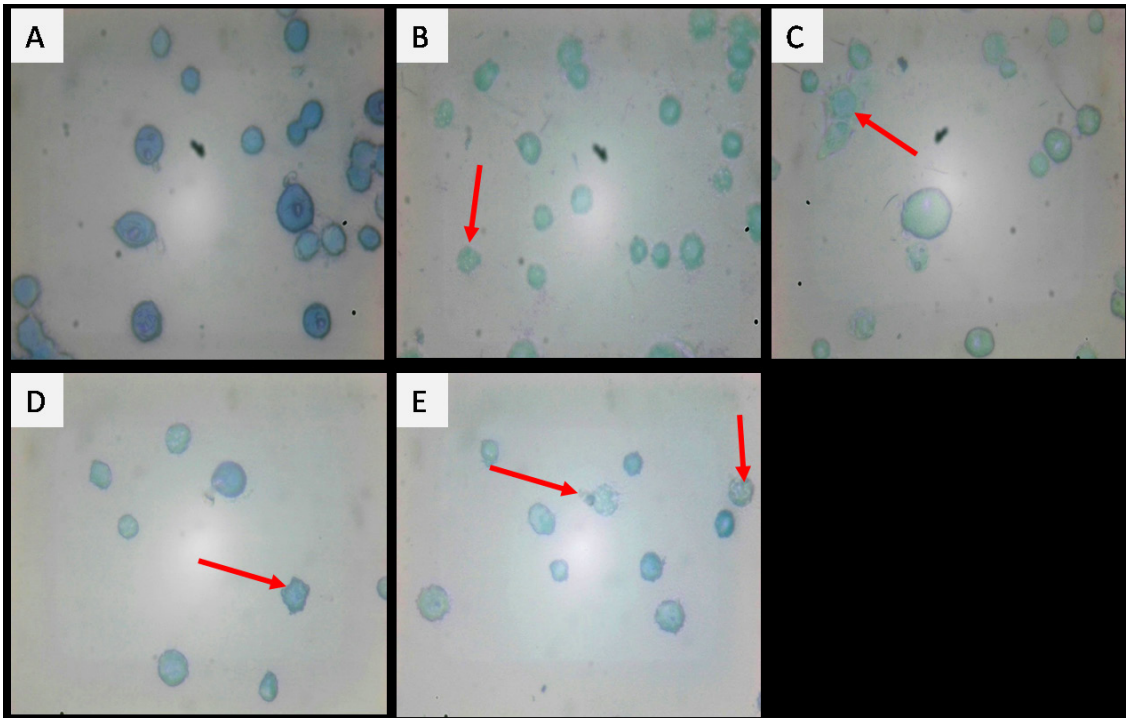


Figure 1. Microphotographs of haemocytes when exposed to magnetic field of strength 0.4493 G showing disintegration. **(A)** Control group **(B)** Exposed for 3 hrs and observed after 24 hrs. **(C)** Exposed for 3 hrs and observed after 48 hrs. **(D)** Exposed for 6 hrs and observed after 24 hrs. **(E)** exposed for 3 hrs and observed after 48 hrs. Arrows are used to depict disintegration. Scale -10x.

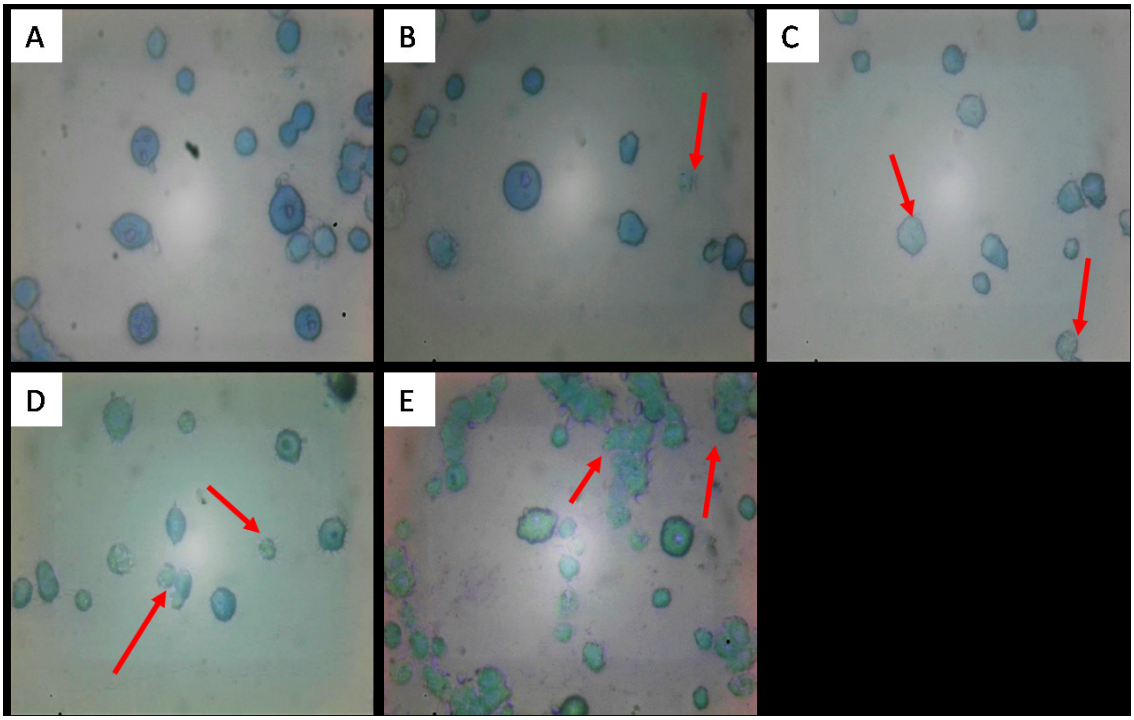


Figure 2. Microphotographs of haemocytes when exposed to magnetic field of strength 0.4493 G. **(A)** Control group. **(B)** Exposed for 3 hrs and observed after 24 hrs. **(C)** Exposed for 3 hrs and observed after 48 hrs. **(D)** Exposed for 6 hrs and observed after 24 hrs. **(E)** Exposed for 3 hrs and observed after 48 hrs. Arrows are used to depict disintegration of haemocytes. Scale – 10X.

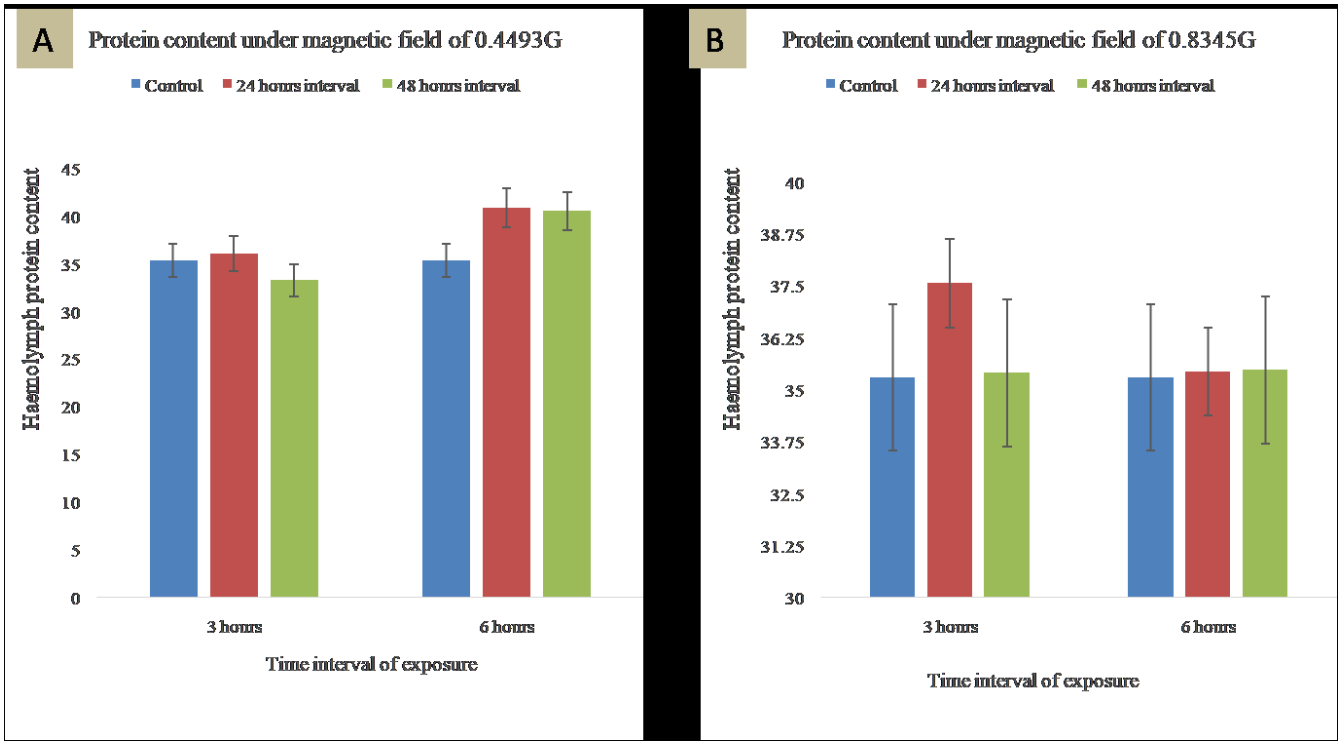


Figure 3. Statuses of haemolymph protein content after exposure to magnetic field of strength **(A)** 0.4493G and **(B)** 0.8345G in control and exposed Eri silkworm larvae.

4. Discussion

The geomagnetic field operating on earth is one of the very important physical forces responsible for sustaining life on earth. With modernization and urbanization, certain aspects have changed, which has led to an imbalance in nature, including alterations in the geomagnetic field. Any imbalance in these physical forces is bound to interfere in normal functioning of the living world, no matter how small those changes maybe, bringing with it larger impacts on the components of the natural ecosystems. This study conducted with Eri silkworm as a model organism evidently signify an effect of higher strength magnetic fields on the haemocytic populations and protein content of exposed *P. ricini* larvae. With an increase in magnetic field strength, a decrease in haemocyte population is a clear indication of the effect of higher magnetic fields on the haemocytes of the larvae suggesting that an increase in magnetic field do have a deleterious effect on insect tissues. These results are consistent with the findings of Elyamani (2020), which shows that magnetic field has definite effects on various biochemical aspects of silkworm[22].

Many studies on the effect of high magnetic fields on insect behavior and their body tissues, viz., perception of gravity by bees and Drosophila, the influence of magnetic field on *Drosophila melanogaster* development by Ramirez *et al.*, also provide similar results[23,24]. They reported that the mortality of eggs and lower viability of eggs with increased field strength abounds entomological literature. Likewise, both the sexes of *D. melanogaster* showed a decrease in wing size under higher magnetic field exposure[25]. However, it was also proved that strong magnetic fields have an influence on cell functions such as ion- channel disruptor[26]. Entomological literature regarding the effect of magnetic fields on insect haemocyte population is very scarce. Nevertheless, the decreased THC can be attributed to the fact that, after exposure to higher magnetic fields the haemocytes disintegrate. This disintegration of the cells accounted for the lower count of THC in the treated worms. Our analysis of the haemocyte profile clearly shows that haemocyte disintegration does occur after exposure to higher magnetic fields. This amply supports our observations on decreasing trend of THC in treated worms.

Regarding the observed changes in haemolymph protein contents in treated worms, the works of Madhuri *et al.*, and Tripathi can be cited in support[27,28]. They reported that insects exposed to higher magnetic field recorded higher protein contents. Madhuri *et al.*, reported that the magnetization of *B. mori* larvae influenced the level of total protein content in their haemolymph; higher the magnetic field (to a certain extent) more the total haemolymph protein content[27]. Similarly, Tripathi also reported an increase in haemolymph protein content in larval haemolymph on exposure to magnetic fields with variations[27,28]. According to their reports it was due to enhanced protein metabolism in the treated insects. They suggested that magnetic fields affect the biosynthesis and utilization of free amino acids for protein metabolism. The effect of magnetic field on protein metabolism in insect as reported by Alexander and Ganeshan[28] also align with the results obtained from our study suggesting the influence of strong magnetic field on biological parameters of organisms[29]. Mathur and Subba, Hsu and Wang, Chitra and Sridhar and Taha reported similar findings on the effect of strong magnetic fields on the amino acid content as well as haemolymph content in silkworm[14,29-32].

However, in our result, although protein percentages showed a gradual increase with increased magnetic field, it was not significantly different to draw any serious conclusion. But we may tentatively suggest that, the slight increase observed may be due to dissolution of the constituent proteins from the disintegrated haemocytes in the haemolymph.

5. Conclusion

From the study we can tentatively suggest that presence of higher magnetic fields do exert some effect on certain physiological parameter of Eri-silkworms. Higher magnetic fields seem to induce changes in haemograms of the larvae. Disintegration of haemocytes and alterations in their haemolymph protein contents has provided promising and encouraging results. These results can be linked to previous assumptions that exposure of strong magnetic fields, which are higher than living organisms are accustomed to, can have toxic effects leading to severe complications. Such information provides encouraging ideas for future use of higher magnetic fields in pest control measures. However, for that, further in-depth studies are of immediate importance.

6. Acknowledgements

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7. Authors' Contribution

Sunayan Bardoloi conceptualized and supervised the study, Bidisha R. Das and Shibani Kalita performed the experiments with the help of Smritimala Sarmah, who set helped in setting up the experiment. Sanghamitra Saharia analysed the data, and Anjumani Ojah edited the draft. Shibani Kalita wrote the first draft.

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