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Enhancing silk production: Exploring the influence of vitamin e on the reproductive performance of *Bombyx Mori L.*

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Abstract
The reproductive performance of *Bombyx mori* silkworms is crucial in sericulture, directly impacting silk production and quality. Antioxidants like Vitamin E are known to enhance physiological functions by reducing oxidative stress and supporting cellular health. This study examines the effects of Vitamin E supplementation on the reproductive performance of *Bombyx mori*. Silkworms were divided into control and treatment groups, with the latter receiving different concentrations of Vitamin E in their diet. Key reproductive parameters, including fecundity, egg hatchability, and larval survival rate were evaluated. The results showed a notable increase in fertility, egg viability, and overall reproductive efficiency in Vitamin E-treated silkworms compared to the control group. This outcome can be the effect of tocopherol, which helps to increase fecundity and fertility. These findings highlight the potential of Vitamin E supplementation could improve reproductive success, benefiting large-scale sericulture by enhancing silk production. Further research is needed to determine the optimal dosage for maximum effectiveness.

Keywords: Vitamin e, silkworm, fecundity, fertility, silkworm, tocopherol

Introduction

Silkworm is considered as a valuable economic important insect which fabricates silk thread in the form of cocoon around itself (Hussain *et al.,* 2011) [7]. The healthy and lustrous silk fibre is appreciated across the globe and has significant unending demand. Therefore, in order to achieve quality silk, the nutritional factor in mulberry plays an important role. Fortification of mulberry leaves with additional supplements has been reported to enhanced silk and cocoon productivity in various studies (Kamala and Karthikeyan, 2019) [8].

In addition to this, the quality and disease-free silkworm eggs are an important parameter to achieve productivity and quality of silk. Fecundity and fertility are the two important factors that affect the physiology of silkworm (Gaurav *et al.*, 2015) [4].

Silkworm eggs are the backbone of silk industry as quality silkworm seeds are basic requisite for conducive growth of silkworm and quality of the silk (Hemmatabadi *et al.,* 2016) [6].

Quality silkworm seeds denotes to the good number of eggs, richness of laying, viability of eggs, hatching uniformity, disease free silkworm eggs and more prominently good rearing performance of the progeny (Hussain *et al*., 2011; Gaurav *et al.*, 2015; Batham and Yadav, 2015) [1, 4, 7].

Vitamins are important part of our diet that are needed only in minute quantity and are essential for the normal growth and maintenance of health as they participate in specific metabolic reactions within the cell (Kamala and Karthikeyan, 2019) [8].

In 1992, Vitamin E also known as tocopherol was first discovered by Evans and Bishop (Chen *et al.,* 2020) [2] as a substance necessary for reproduction afterwards, Vitamins E has comprehensively been studied, as a result it has become well known powerful lipid-soluble antioxidant (Mutalip *et al.*, 2018) [10] to protect the reproductive system (Zubair, 2017) [17].

Vitamin E can efficiently reverse the dreadful impact by oxidative stress brought to the reproductive system and endocrine system, and is extensively used in the field of reproductive medicine (Chen *et al.,* 2020) [2]. This fact has evidently been supported by various studies conducted on different animal models. For instance, in a study it has been showed that moderate amount of Vitamin E in poultry diet remarkably protected eggs qualities in female birds and semen/sperm qualities in male birds through reducing the lipid per oxidation in eggs and semen/sperms (Rengaraj and Hong, 2015) [12]. By considering above result the proposed study was conducted on silkworm to achieve fertility, fecundity and vitality of the eggs.

Methodology

The present study utilized the bivoltine silkworm double hybrids. These selected races were reared following the standard rearing methods recommended by Dandin and Giridhar (2010) [3].

Vitamin E Supplementation: An experiment was carried out during the 4th and 5th instar stages of silkworm development. For the study, 100 healthy larvae were placed in each tray, and the experiment was replicated three times, including a control group. The test larvae were fed mulberry leaves treated with Vitamin E. To minimize any direct odor that could affect the silkworms, Vitamin E was applied to the dorsal surface of the leaves. Two experimental batches were established alongside the control. In the first batch, silkworms received Vitamin E-supplemented leaves starting from the 4th instar stage, while in the second batch; supplementation began only at the 5th instar. During the 4th instar, the treated leaves were given twice daily, whereas in the 5th instar, they were provided once daily.

Mating duration: Moths from each pairing were permitted to mate for duration of 5 hours. After the designated mating time, the pairs were separated, with female moths placed for oviposition and the males discarded. The entire procedure was carried out under controlled conditions of 25 ± 1 °C temperature and 75%–80% relative humidity. Each treatment was replicated three times.

Fig 1: Mating

Fig 2: Mating of moths undergone different treatments

Egg count: The traditional method for counting silkworm eggs involves using an ink or sketch pen. The egg sheet is covered with a transparent sheet of paper, and the eggs from a single DFL are counted by marking them with the pen. Each DFL contains between 450 and 550 eggs. The total egg count for all the DFLs on the sheet is calculated by multiplying the number of eggs in one DFL by the total number of DFLs on the page. This process is done manually and is quite time-consuming (Kawade *et al.*, 2014) [9].

Egg Vitality: Fertilized eggs develop a distinct color and can be differentiated from unfertilized ones about 48 hours after oviposition. The pigment 'ommochrome' spreads through the serosal cuticle after the formation of the amnion and serosal layers, which occurs as a result of fertilization. This pigment gives the eggs a deep, dark brown color (Singh *et al.*, 2002) [16].

In unfertilized eggs, the cuticle remains unchanged, and they retain the color they acquired at oviposition. Non-diapausing eggs, however, do not have the ommochrome pigment, making it hard to tell fertilized eggs apart from unfertilized ones. This difference becomes noticeable only once the eggs reach the pin-head stage in development. When the embryos fully mature, the sclerotization of the cuticular layers causes unfertilized eggs to retain their original color, while fertilized eggs turn blue (Saheb *et al.*, 2009) [13].

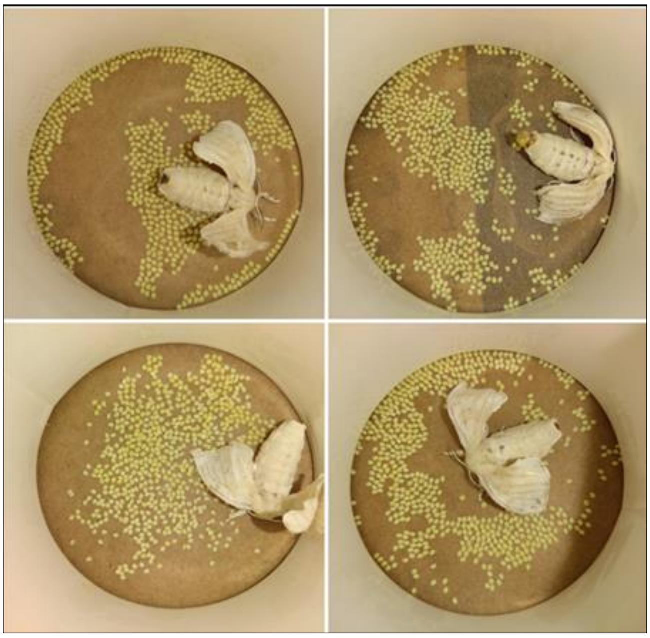


Fig 3: Eggs laid by female moths

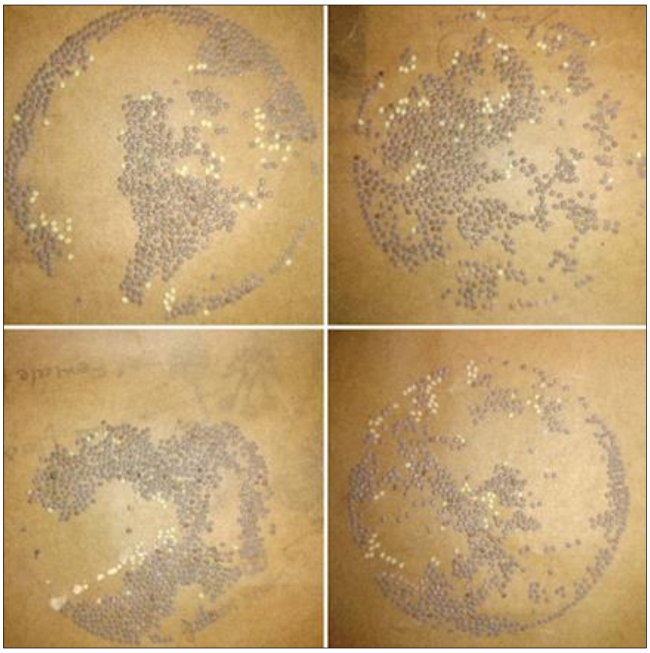


Fig 4. Fertilized and unfertilized eggs

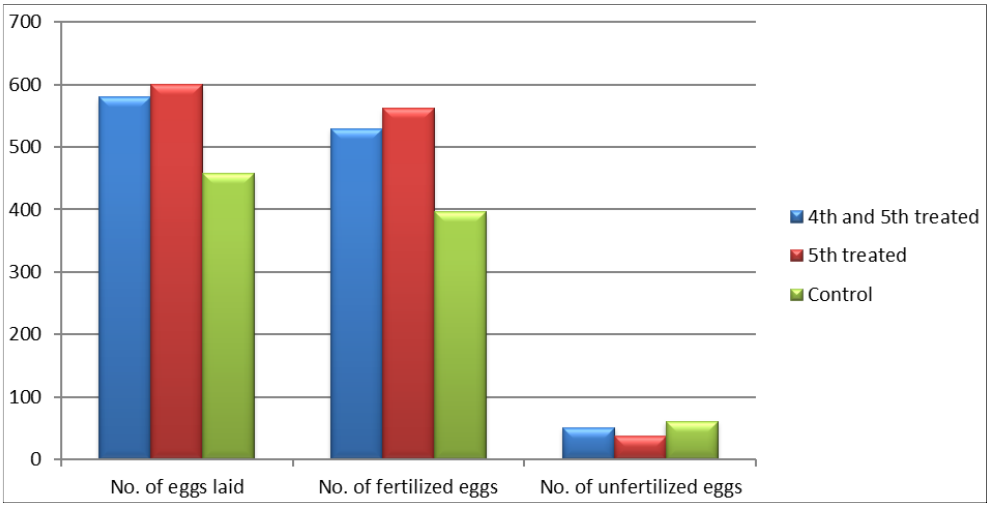
Results

Fecundity: It has been clear from the experiment that treatment, particularly during the 5th instar, significantly enhances fecundity as compared to the other two groups. The highest no. of eggs laid was in 5th treated group 599.667; it was then followed by 579.667 and 457 in 4th and 5th treated and the control group respectively. As a result of all the combinations, it has been discovered that Vitamin E treatment exerts significant influence on total fecundity, and have a substantial effect on egg fertility same as found by Sarkar *et al.*, 2009 [14].

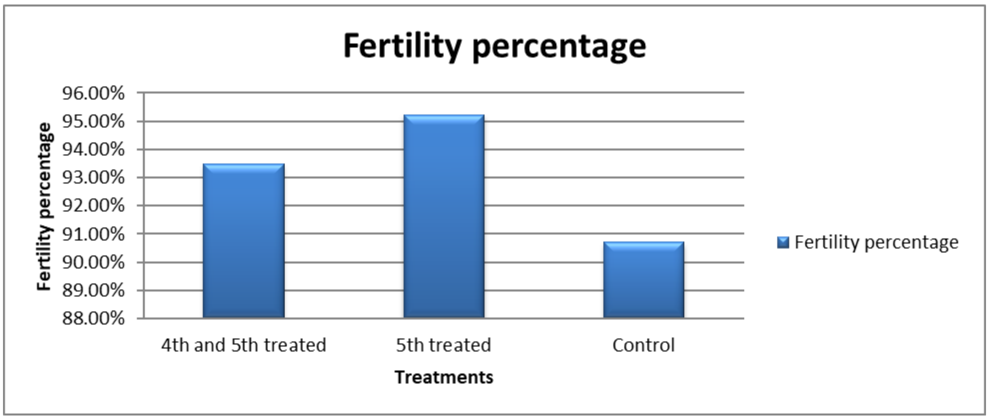
Fertility: From the experiment it has been clear that treatment, particularly during the 5th instar, significantly enhances fecundity as compared to the other two groups. The highest fertility was shown in 5th treated group 95.23% whereas the lowest was found in the control group 90.71%. Based on the result it has been discovered that Vitamin E treated group showed higher rate of fertility when compared to the controlled one same as found by Sarkar *et al.,* 2009 [14].

Table 1: Effect of Vitamin E treated in 4th and 5th instar on fecundity and fertility of moth.

Treatments	No. of eggs laid	No. of fertilized eggs	No. of unfertilized eggs	Fertility percentage
4th and 5th treated	579.667	529	50.667	93.49%
5th treated	599.667	562	37.667	95.23%
Control	457	596	61	90.71%
'F' Cal. value	7.235751	9.06259	3.319245	7.479925
Probability (p)	0.025177	0.015383	0.106996	0.023458
'F' Tab. value@0.05	5.143253	5.143253	5.143253	5.143253



Graph 1: Showing the effect of different treatments of Vitamin E along with controlled one on the fecundity and egg count of the moth



Graph 2: Showing the effect of different treatments of Vitamin E along with controlled one on the fertility of the moth.

Discussions

The result of current study clearly indicate that Vitamin E treated silk moth achieve better fertility and fecundity as compared to the control. This result may be due to tocopherol which is beneficial in boosting the fertility and fecundity. Present result is well supported by the studies conducted by Ito in 1978. Vitamin E was discovered to be quite beneficial in enhancing moth fertility. According to some previous studies, vitamin E has been used as a significant component in artificial feeds for the rearing of insects (Pal and Datta 2002) [11]. Siddiqui and Swarup (1980) [15] found that artificial diets deficient in vitamin E resulted in fewer eggs being laid by *Chilo partellus* (swinhoe) moths. According to Gillot and Friedel (1977) [5], the production of eggs is affected by certain substances secreted by male insects during mating that enhance female fecundity. The addition of Vitamin E to the diet resulted in an increase in egg laying. As a result of this study and previous publications, it can be concluded that supplementing mulberry leaves with vitamin E is very beneficial in enhancing the quality of eggs. In future Vitamin E supplement silkworm could be used as a suitable option to improve overall fecundity and fertility of silkworm thereby; it would be a great boost to silkworm industry. Thus in future, industries can use the vitamin to enhance the quality of end product.

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