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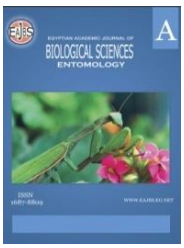

The Effect of Fortificated Mulberry Leaves with *Echinacea Purpurea* on Silkworm (*Bombyx mori* L.) Physiology and Production

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ABSTRACT

Mulberry silkworm is more sensitive to any infection during the rearing season. Maintaining a healthy larva was the main target of this research to lower the probability of infection with microbes besides increasing silk production. There was evidence-based efficacy suggesting that *Echinacea purpurea* can help to improve immune system barriers by increasing the activity of antioxidant enzymes such as Peroxidase and Phenol oxidase, which boosts silk production. The present investigation was carried out to study the impact of *Echinacea purpurea* on some biological and biochemical parameters. Silkworm larvae reared on mulberry leaves enriched with different concentrations of Echinacea purpurea (0.5%, 1%, and 2%) at the beginning of the 4th instar till the spinning stage. The obtained results indicated that the feeding larvae on 2% concentration of Echinacea purpurea extract increases, all the economic and biological characteristics also, most of the biochemical parameters (total protein, peroxidase activity, and phenoloxidase). while larvae fed at 1% concentration recorded high ascorbic acid values.

INTRODUCTION

Silkworm *Bombyx mori* L. has been domesticated in Egypt for more than 100 years. Due to its sensitivity, it is subjected to some bacterial and viral infections that cause loss of cocoon production, which affects an expected income for farmers or even youth who want to have a project generate an income return. Having information about silkworm's nutrition is essential for determining the health and growth of silkworm larvae and their cocoon parameters. Feeding with nutritionally enriched leaves has a positive impact on the quality and quantity of silk produced. Conversely, any nutritional deficits in the silkworm's diet can affect their health and subsequently affect the quality of silk produced. One of the most important supplements that confuse any deficient in the silkworm diet is the botanical extract due to its secondary metabolites that affect silkworm physiology Etebari and Matindoost (2004)

All over the world, there are no silkworm types resistant to bacterial infection or pests. Therefore, during the rearing period, we added some additives that have an immune power and also, increase the production. Recently, mulberry leaves have been fortified

with plant extracts to boost production (Sujatha and Purushotham, 2003; Kuntamalla and Akkinapally, 2004 and Takhlique, 2012).

Also, mulberry leaves play a vital role in the silk production. To improve the economic parameters of the silkworm, it must feed on nutritional leaves (Khezrian *et al.*,2021). The new technique in silkworm rearing to increase their performance and economic parameters is feeding them on enhanced mulberry leaves with supplementary nutrients (Etebari and Matindoost, 2005). Vitamins, amino acids, and proteins that added to mulberry leaves to increase their nutritional efficiency and improve the silkworm's economic traits (Singh, *et al.,* 2005; Rani and Singh, 2011). *Echinacea purpurea* is one of the most important medicinal plants. Studies revealed *Echinacea* extracts have alkamides that have immunomodulatory properties in vitro and in vivo. (Goel *et al.,* 2002; Gertsch *et al.,* 2004). Also, it contains polysaccharides that inhibit cell apoptosis and oxidant stress (Vimalanathan *et al.,* 2017; Hou *et al.,* 2020). *Echinacea* extract might be able to boost the function of monocytes and natural killer cells, which are essential components of the body's immune system and help fight off infections (Declerck *et al.,* 2021).

Proteins are the major component of the hemolymph plasma. Protein-rich mulberry leaves are responsible for silk production. As well, as a protease enzyme that is responsible for the conversion of mulberry protein to silk protein (Kumar and Kalpana, 2009). Phenol oxidase plays a vital role in the defence mechanism of invertebrates (Liu *et al.,* 2007).

The main role of the peroxidase enzyme is to protect the organism from oxidation stress. Damaged cell caused by free radicals is prevented by the presence of peroxidase activity that has immune function (Sun *et al.,* 2009).

Ascorbic acid, also known as vitamin C, is a water-soluble vitamin with the chemical formula C6H6. Despite not having a fully known function yet, it plays a role in tyrosine metabolism, steroid synthesis, and carotenoid synthesis, as well as acting as a feeding stimulant immunity and detoxifications. It possesses antioxidant power and is a potential phage stimulant. Additionally, possess a growth promoter and a booster of silk production in silkworms (Javed and Gondal 2002; Hussain and Javed 2002; Prassad 2004; Etebari *et al.,* 2004; Etebari and Matindoost, 2005).

Our investigation studies the effect of *Echinacea purpurea* supplemented with mulberry leaves on silkworm production and immunity.

MATERIALS AND METHODS

The investigation was carried out at the Sericulture Research Department in Plant Protection Research Institute, Agricultural Research Center.

Silkworm:

Silkworm seeds (F_1X_7) were imported from China.

Plant Extract Preparation:

Echinacea purpurea shrubs were purchased from IMTENAN shop for medical and healthy products. Dried and ground using an electric grinder: 500 g of plant powder was added into the flask of a Soxhlet apparatus along with 400 ml of 70% ethanol. The apparatus was run for 12 h and thereafter the flask containing active plant hydroalcoholic extracts was discharged in a desiccator to dry off in vacuum condition. Then, the extract was preserved at −20 °C (Harikrishnan *et al.,*2010).

Experimental Design.

Young larvae (1st~3rd instars) were reared at 27~28 °C and 85%~90% relative humidity, and the late-age larvae (4^{th} and 5^{th} instars) were maintained at 24~26 °C with a relative humidity of 70%~80%. Rearing was carried out under hygienic conditions

according to (Marwa 2020). At the beginning of the 4th instar, larvae were divided into three groups, each one containing 300 larvae that were divided into three replicates (each one containing 100 larvae). Each group was treated with one concentration of *Echinacea* extract (0.5, 1 and 2%) till spinning.

A- Biological Index:

Larval weight at the end of the 4th and 5th instar, pupation (%), and cocooning percentage (%) were registered.

B- Cocoon Traits.

Fresh cocoon weight(g.), cocoon shell weight (g.), pupa weight (g.) and cocoon shell ratio (%) was calculated according to (Tanaka1964) for both male and female.

$$\text{Silk ratio (\%)} = \frac{\text{Weight of cocoon shell (g.)}}{\text{Weight of fresh cocoon (g.)}} x100$$

C- Biochemical Analysis:

Hemolymph was collected from treated larvae on the 6th day of the 5th larval instar by removal of the thoracic leg in 1.5 ml Eppendorf tubes with a small amount of phenyl thio-urea crystal(PTU) as an anti-coagulant substance (Mahmoud 1988). The tubes were kept at -20°c, and the blood samples were centrifuged at 10000 rpm for 10 minutes at 5°c. the supernatant was assayed to determine.

a- **Phenol Oxidase Activity.** was determined according to a modification of (Ishaaya1971).

b-Peroxidase Activity. was determined according to the procedure given by (Hammerschmidt *et al.*, 1982).

c-Total Proteins were determined by the method of (Bradford 1976).

d- Vitamin C or ascorbic acid method (A.O.A.C 1975) is based on the measurement of the extent to which a 2,6-dichlorophenol-indophenol dye solution is decolorized by the presence of ascorbic acid.

Data Analysis:

Obtained data was analyzed as one-way ANOVA, using Proc ANOVA in SAS (Anonymous 2003) and means were compared by LSD (P= 0.05 level) in the same program.

RESULTS

A- Biological Index:

Table (1) showed that the application *of Echinacea purpurea* to the 4th larval instar recorded significantly, the highest weight for control (0.498g.) significantly, while all treatments recorded less weight on par with each other (0.421, 0.417 and 0.408g.) for (2%, 1%, and 0.5%) respectively. Otherwise, the 5th instar weight was maximum for 2% treatment (2.49g.) with a significant variance among other treatments and control, minimum results were (2.05 and 1.96g.) for (0.5%, and Control) respectively, with no significant variance between each other.

The cocooning percentage showed no significant variance between 2% and 1% treatments (100.00, 98.45%). Also, pupation ratio, data were recorded (100.00 and 100.00) for (2% and 1%) with no significance, respectively. However, all treatments differed from the control significantly, which recorded the lowest value in the previous criteria (78.00 and 81.90 %), respectively.

Table 1: Effect of fortified mulberry leaves with *Echinacea purpurea* on some biological parameters.

Treatment parameter	4th Larval weight (gm.)	5th Larval weight (gm.)	Cocooning Percent%.	Pupation Ratio%
0.5%	0.408 b	2.05 c	91.68 b	96.18 b
1%	0.417 b	2.32 b	98.45 ba	100.00 a
2%	0.421 b	2.49 a	100.00 a	100.00 a
Control	0.498 a	1.96 c	78.00 c	81.90c
P	<0.0001	<0.0001	<0.0001	<0.0001
L.S. D	0.0304	0.127	7.160	2.306

B- Cocoon Traits:

As shown in Table (2) compared with the control group. Feeding on *Echinacea purpurea* increases all cocoon parameters compared with the control group especially the cocoon shell ratio which is an important indicator for evaluating the success of the investigation. It was obvious that feeding on 2% concentration is the most effective that recorded (1.28g., 0.272g., 1.01g., and 21.12%) significantly in females for (C.W., C.S.W., P.W., and C.S.R%) respectively. But, for the male, 2% treatment recorded maximum results in cocoon weight, pupa weight, and cocoon shell ratio compared to other concentrations (0.5% and 1%) whereas, the results registered were (0.993, 0.950 and 0.956g.) for male cocoon weight, (0.748, 0.723 and 0.730) for pupa weight and (23.96%, 23.14 and 23.02%) for cocoon shell ratio for (2%, 1%, and 0.5%) respectively. Cocoon shell weight 2% treatment recorded the highest data significantly (0.238 g.).

Table 2: Effect of fortified mulberry leaves with *Echinacea purpurea* on cocoon traits.

	Female				Male			
	C.W(g.)	**C.S.W(g.)**	**P.W(g.)**	**C.S.R%**	**C.W(g.)**	**C.S.W(g.)**	**P.W(g.)**	**C.S.R%**
0.5%	1.11 b	0.213 b	0.894 b	19.11 bc	0.956 a	0.220 b	0.730 a	23.02 ab
1%	1.13 b	0.230 b	0.909 b	20.18 ab	0.950 a	0.220 b	0.723 ab	23.14 a
2%	1.28 a	0.272 a	1.01 a	21.12 a	0.993 a	0.238 a	0.748 a	23.96a
C	0.945c	0.169 c	0.768 c	17.89 c	0.870 b	0.187 c	0.679 b	21.50 c
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0034
L.S.D	0.0761	0.0172	0.0645	1.134	0.0527	0.0158	0.044	1.387

C.w: Cocoon weight C.S.W: cocoon shell weight P.W: pupa weight C.S.R%: cocoon shell ratio

C- Biochemical Analysis.

Among the different concentrations 2% treatment recorded significantly high results in total protein, peroxidase, and phenol oxidase that were (79.53 mg\ml, 17.66 Δ O.D. /min/ ml, and 13.63 O.D.units/min/ ml) respectively. However, the lowest value was (64.533 mg\ml) in negatively treated control for total protein while, peroxidase and phenol oxidase enzymes recorded the minimum results (7.63 Δ O.D. /min/ ml and 8.30 O.D.units/min/ ml) for 1% treatment respectively, with no significant variance between it and control.

On the other hand, values of larvae fed on mulberry leaves fortified with 1% of *Echinacea purpurea* showed the highest result significantly, (139.66 ug A.A./ ml) while data was registered for larvae fed on *2% concentration* (107.33 ug A.A./ ml) without significance compared to other treatments.

Table 3: Effect of fortified mulberry leaves with *Echinacea Purpurea* on some biochemical analysis.

Treatment parameter	Total Protein (mg/ ml)	Peroxidase (Δ O.D. /min/ ml)	Phenol oxidase (O.D.units/min/ ml)	Ascorbic Acid (ug A.A./ ml
0.5%	76.63 a	8.06 b	8.86 b	123.66 b
1%	68.20 b	7.63 b	8.30 b	139.66a
2%	79.53 a	17.66 a	13.63 a	107.33b
Control	64.53c	7.600 b	8.70 b	111.33 b
P	<.0001	<.0001	<.0001	<.0001
LSD 0.05	2.9305	1.8362	0.837	11.026

DISCUSSION

Nutritional requirements, conversion efficiency and strength immunity of silkworms play a significant role in determining the cost-benefit ratio of rearing them. These factors are crucial for identifying superior breeds of silkworms.

A- Biological and Cocoon Characters:

This present study revealed that feeding silkworm larvae on mulberry leaves enriched with *Echinacea purpurea* only during the 4th larval instar did not affect larval weight as shown previously, these results are in line with (Saad *et al.,* 2014) who referred to the mulberry leaves enriched with food supplements affect significantly the weight of full-grown larvae, silk gland, and cocooning percentage. However, the results took another direction during the 5th instar, the larval weight increased, cocooning and pupation ratio also, increased, significantly.

These findings are in agreement with (Shinde *et al*., 2014) who proved that the important factors affecting on silk industry are mulberry variety and nutritive additives. Also, (Etebari *et al.,* 2004) referred to nutritional supplements, such as proteins, carbohydrates, vitamins, minerals, hormones, and antibiotics, which can enhance larval weight, pupal rate, and cocoon weight in silkworm larvae. Nutrient quality of mulberry leaves has a significant impact on biomass gain and economic traits. *E. purpurea* extract has a positive impact on all economic parameters of silkworms. (Khezrian *et al.*,2021) in conformity with our results, many botanical extracts (*Echinacea purpurea*) have been impacted by silkworm economic characteristics. (Saravanan *et al.*,2011) , Changes in silkworm larval weight when different feeding supplements were used might have been due to the increase in protein production. Also, (Guiqin *et al.*, 2020) who studied the effect of flavonoids as secondary metabolites on silkworm performance, found that, a positive impact on the activity and silk production. *Cucurbita moschata* extract boosts silkworm growth and increases larval weight, cocoon, shell weight also cocoon shell ratio at higher concentrations (Jamela *et al.,* 2023). 10% of *C. longa* extract has a positive impact on all cocoon traits and economic characteristics of mulberry silkworms (Rajitha and Savithri 2015). Some medicinal plant extracts (*A. vasica, B. spectabilis, P. niruri, T. arjuna* and *Pongamia glabra)* enhance all biological and cocoon traits of silkworm larvae after feeding during 4th and 5th larval instars (Latha *et al.*,2011).

B- Biochemical Analysis:

The increase in hemolymph total protein indicating to an increase in digestive activities that lead to protein accumulation and transport by heamolymph to other tissues. These findings go in line with (Nagata and Kobayashi 1990) refer to the elevations of hemolymph protein during the 5th instar due to the active secretion of proteins by other tissues. (Suramya *et al.,* 2020) who proved that *Echinacea* is a potent anti-oxidant, phage stimulant, growth promoter, and silk production booster. Also, (Marwa 2020) goes in our

line feeding silkworms on 2.5% of *Aleo vera* increase total protein. *Echinacea* contains caffeic and ferulic acid derivatives, caftaric acid, chicoric acid, and chlorogenic acid as major phenolic compounds, along with polysaccharides and glycoproteins that, explain the elevation of peroxidase, and phenol oxidase activities in larvae fed on mulberry leaves fortified with 2% concentration *extract* as shown in previous results. *Echinacea* enhances immune function in both healthy and immuno-compromised animals (Mariangela *et al.*, 2018). Polysaccharides protect the body by preventing the absorption of free radical species and safeguarding them from oxidation before they can be absorbed. the addition of 1 g. of polysaccharides boosted peroxidase activity (Chang and Chen 2003). Also, (Fu *et al*.,2007) revealed that the ingestion of polysaccharides obtained from *Utricularia rigida*, a type of seaweed, can play a crucial role in preventing the deterioration of antioxidant defence. (Manjula *et al.*,2020) phenoloxidase enzyme increased after feeding *S. litura* on P.B of *Manihot esculenta* leaf extract containing phenolic compounds. 2.5% of *Aleo vera* increase peroxidase activity in silkworm heamolymph as reported by (Marwa 2020).

On the other hand, larvae fed on mulberry leaves supplemented with *1% of Echinacea purpurea extract* only recorded high ascorbic acid levels, which declined gradually in treatments fed on 2% and 0.5% without significance. This result may be due to the increasing of *Echinacea purpurea* dose that contains polysaccharides and antioxidants, which are in conformity with (Lamikanra and Watson 2001) who referred to the inverse relation between ascorbic acid and peroxidase activity. However, the total protein level in the same group (1%) was low compared with the 2% treatment group. The result of increasing ascorbic acid leads to an increase in blood glucose which decreases stimulation of the hunger center decreases feed intake and affects silk production. These results are supported by Etebari *et al.* (2004) findings, The act of providing silkworms with excessive quantities of ascorbic acid has been found to result in hypervitaminosis, leading to a decrease in their overall weight that leads to silk reduction.

CONCLUSION:

The target of this study is finding an extract that has double power, and effect depending on its concentration: activate antioxidant enzymes to reduce infection and enhance silk production. Also, despite the positivity of a 1% concentration (which contains a high amount of ascorbic acid), on immunity and that prevents cell apoptosis, a high (2%) concentration of *Echinacea purpurea* also has flavonoids and phenolic compounds that raise antioxidant enzymes. Therefore, may be the most effective concentration (2%) increases total protein, phenol oxidase, and peroxidase enzymes., The other two concentrations (0.5%, 1%) are responsible for increasing immunity which reduces pathogenic invasion, which is affecting silk production.

REFERENCES

A.O.A.C. (1975). Ascoric acid. In analysis of fruit and vegetable products. 94-101.

Anonymous. (2003). SAS Statistics and graphics guide, release 9.1. SAS Institute, Cary, North Carolina 27513, USA.

Bradford, M.M. (I976). A rapid and sensitive method for the quantitation of microgram quantities of proteins utilizing the principle of protein-dye binding. *Analytical Biochemistry*,72:248-254.

Chang, M. and Chen, X. (2003). Effect of two kinds of polysaccharide on the phenoloxidase activity in macrobrachium nipponensis. *Acta Hydrobiologica Sinica,* 27: 269–272.

Declerck, K., Novo, C.P., Grielens, L., Camp, G.V., Suter, A. and Berghe, W.V. (2021).

Echinacea purpurea (L.) Moench treatment of monocytes promotes tonic interferon signaling, increased innate immunity gene expression and DNA repeat hypermethylated silencing of endogenous retroviral sequences. *BMC Complementary Medicine and Therapies*, 21:141.

Etebari, K. and Matindoost, L. (2004). Effects of hypervitaminosis of vitamin B3 on silkworm biology. *Journal Bioscience,* 29(4): 417-422.

Etebari, K. and Matindoost, L. (2005). Application of multi-vitamins as supplementary nutrients on biological and economical characteristics of silkworm *Bombyx mori* L. *Journal of Asia-Pacific Entomology,* 8:107–112.

Etebari, K. and Matindoost, L. (2005). The effect of vitamin C on biological, biochemical and economical characteristics of the silkworm *Bombyx mori* L. *journal Asia-Pacific Entomology,* 8(1):107- 112.

Etebari, K., Ebadi, R. and Matindoost, l. (2004). Effect of feeding mulberry's enriched leaves with ascorbic acid on some biological, biochemical and economical characteristics of silkworm *Bombyx mori*.L. *International Journal Industerial Entomology,* 8: 81-87.

Fu, Y. W., Hou, W.Y., Yeh, S.T., Li, C.H. and Chen, J.C. (2007). The immuno-stimulatory effects of hot-water extract of *Gelidium amansii* via immersion, injection and dietary administrations on white shrimp *Litopenaeus vannamei* and its resistance against *Vibrio alginolyticus*. *Fish and Shellfish Immunology,* 22(6):673–685.

Gertsch, J., Schoop, R., Kuenzle, U. and Suter, A. (2004). *Echinacea* alkylamides modulate TNF-alpha gene expression via cannabinoid receptor CB2 and multiple signal transduction pathways. *FEBS Letters,* 9: 577-563.

Goel, V., Chang, C., Slama, J.V., Barton, R., Bauer, R. and Gahler, R. (2002). Alkylamide of *Echinacea purpurea* stimulate alveolar macrophage function in normal rats. *International Immunopharmacology,* 2:381–387.

Guiqin, S., Kang, Z., Ren, F., Zhou, Y. and Guo, P. (2020). Effects of Quercetin on the Growth and Expression of Immune-Pathway-Related Genes in Silkworm (Lepidoptera: Bombycidae). *Journal of Insect Science,* 20,6 (23): 1–9.

Hammerschmidt, R., Nuckles, F. and Kuc, J. (1982). Association of enhanced peroxidase activity with induced systemic resistance of cucumber to Colletotrchum lagenarium. *Physiological Plant Pathology,* 20:73-82.

Harikrishnan, R., Balasundaram, C. and Heo, M.S. (2010). *Lactobacillus sakei* BK19 enriched diet enhances the immunity status and disease resistance to *streptococcosis* infection in kelp grouper, *Epinephelus bruneus*. *Fish & Shellfish Immunology,* 29:1037–1043.

Hou, T., Xu, Q., Li, F.,Yang, C., Wang, T., Huang, Z. and Hao.(2020). Polysaccharide from *Echinacea purpurea* reduce the oxidant stress in vitro and in vivo. *international journal of Biology and Macromolecules*, 149: 41–50.

Hussain, M. and Javed, H. (2002). Effect of 0.2% N with various combinations of ascorbic acid on growth and silk production of silkworm, *Bombyx mori. Asian Journal of Plant Science,* 1(6):650-651.

Ishaaya, I. (1971). In the armored scale Aonidiella aurantii and Observation on the phenoloxidase system *Chrysomphalus aonidum*. *Comparative Biochemistry Physiology*, 39: 935-943.

Jamela, S.M. M., Karnan, P., Devi, C.M. and Kumar, R.S.P. (2023). Effect of Herbal Plant Extracts on the Economic Parameters of Silkworm *Bombyx mori* L. *Inti Journal*, Vol.2023:30 eISSN:2600-7320

Javed, H. and Gondal,M.H. (2002).Effect of food supplementation by Ascorbic acid on larval mortality of silkworm *Bombyx mori* L. *Asian Journal Plant Science*,1:56-

557.

Khezrian, A., Bagheri, M., Sourati, Z. R., Kheirkhah, R. Y., Nematollahian, S. and Zahmatkesh, A. (2021). The effects of feed supplements and *Nosema bombycis* infection on economical traits in various silkworm breeding lines. *Entomological Experimental Applied*,170:277–283.

Kumar, M. N. A. and Kalpana, G. V. (2009). Studies on the seasonal variation in the protease activity in different developmental stages of productive and robust silkworm breeds and their hybrids of *Bombyx mori* L. *National Journal of Life Sciences*, 6 (1):113-119, ref.12.

Kuntamalla, S. and Akkinapally, P. R. (2004). Effect of botanical extract on Post cocoon characters and in management of flacherie disease in silk worm *B. mori. Biochemical cell, Archiev*, 4 (2):135-142.

Lamikanra, O. and Watson, M. A. (2001). Effects of ascorbic acid on peroxidase and polyphenoloxidase activities in fresh-cut cantaloupe melon. *Journal of Food Science*, 66:1283–1286.

Latha, S., Bhaskar, R.N. and Pallavi, C. (2011). Effect of botanical treatment to *Bm*NPV polyhedral bodies on cocoon parameters of silkworm B. mori (PM x CSR2). *International Journal Advanced Biology*,1:45-51.

Liu, H., Jiravanichpaisal, P., Cerenius, L. Lee, B.L. and Söderhäll, I. (2007).Phenoloxidase is an important component of defense against Aeromonas hydrophilla infection in a Crustacean *Pacifastacus leniusculus*. *Journal of Biological Chemistry*, 282:33593-33598.

Mahmoud S. M., (1988). Activation of Silk Secretion by Silkworms, *Philosamia ricini* and *Bombyx mori* L. After Applying Antibiotics. Ph. D. Thesis, Faculty of Agriculture, Cairo Univ.

Manjula, P.,Lalitha, K.,Vengateswari, G., Patil, J., Senthil, S.N. and Subramanian, M.S. (2020). Effect of *Manihot esculenta* (Crantz) leaf extracts on antioxidant and immune system of *Spodoptera litura* (Lepidoptera: Noctuidae). *Biocatalysis and Agricultural Biotechnology*, 23:101476.

Mariangela, R., Alessandra, M., Silvia, L., Ilaria, A., Antonella, R., Pietro, A., Milena, A. F., Gabriella, P., Mara, N. and Simone, P. (2018). The Pivotal Role of Vitamin D, Vitamin C, Zinc, and *Echinacea* in Three Main Immune Interactive Clusters (Physical Barriers, Innate and Adaptive Immunity) Involved during an Episode of Common Colds Practical Advice on Dosages and on the Time to Take These Nutrients/Botanicals in order to Prevent or Treat Common Colds. *Evidence-Based Complementary and Alternative Medicine*. 36.

Marwa, N. M. (2020). Improving the silk industry by studying the effect of several aqueous extracts to enhance the efficiency of silkworm *Bombyx mori* L. Production. *Journal of Plant Protection and Pathology*, Mansoura Univ., 11 (2): 121-126.

Nagata, M. and Kobayashi, M. (1990). Quantitative changes in storage proteins during larval development of silkworm, *Bombyx mori*. *Journal of Sericulture Science Japan*, 59(6): 461-468.

Prassad, P. R. (2004). Effect of fortification of ascorbic acid through mulberry leaf on cocoon traits of Pure Mysore race of silkworm, *Bombyx mori* L. *Indian Journal Entomology*, 66: 37-39.

Rajitha, K. and Savithri,G. (2015). Studies on symptomological and economic parameters of silk cocoons of *Bombyx mori* inoculated with *Beauveria bassiana* (Bals.). *Vuill. International Journal Current Microbiology Research Applied Science*, 492:44-54.

Rani, A. and Singh, R.(2011). Probiotic supplementations to improve commercial characteristics, disease resistance and protein In the silkworm *Bombyx mori* L. *World Journal of Biological Research.* 4:12–26.

Saad, I., Taha, H. and Saad, I. (2014). Effect of mulberry leaves enriched with the amino acid glycine on some biological aspects of the silkworm, *Bombyx mori* L. *Minufiya Journal Agriculture Research,* 39(2):759-769.

Saravanan, M., Vemu, A.K. and Barik, S.K. (2011). Rapid biosynthesis of silver nanoparticles from *Bacillus megaterium* (NCIM 2326) and their antibacterial activity on multi drug resistant clinical pathogens. *Colloids and Surfaces B: Biointerfaces*, 88: 325–331.

Shinde, K., Avhad, S. and Hiware, C. (2014). Impact of spacing's and fertilizers on the production of M5 Mulberry variety. *International Journal of Interdisciplinary Multidisciplinary Studies,* 1: 344–348.

Singh, K., Chauhan, R., Pande, A., Gokhale, S. and Hegde, N. (2005). Effect of use of *Lactobacillus plantarum* as a probiotic to improve cocoon production of mulberry silkworm *Bombyx mori* (L). *Journal of Basic and Applied Sciences,* 1:1–8.

Sujatha, K. and Purushotham, A.(2003). Effect of Curucuma Longa on the commercial characters of multivoline silk worm. *Journa Experimental Zoology India,* 6 (1):109-111.

Sun, F., Zhou, Q., Wang, M. and An, J. (2009). Joint stress of copper and petroleum hydrocarbons on the polychaete Perinereis aibuhitensis at biochemical levels. *Ecotoxicology and environmental safety*, 72(7): 1887-1892.

Suramya, W., Jessica, P., Tim, C., Esra, M., Brian, B., Cynthia, V. R. and Kristen, R.A. (2020). Strategy for test article selection and phytochemical characterization of *Echinacea purpurea* extract for safety testing Food. *Chemistry and Toxicology,*137:111-125.

Takhlique, M. D. (2012). Medicinal plant extracts and their valuable effects on various parameters of silk worm hybrid (PMxCSR2). *International Journal of Green and Herbal Chemistry*,1 (3): 391-394.

Tanaka Y (1964) Sericology central silk board, Bombay (95). B Megdoot Marine Drive:216–220.

Vimalanathan, S., Schoop, R., Suter, A. and Hudson, J. (2017). Prevention of influenza virus induced bacterial super infection by standardized *Echinacea purpurea*, via regulation of surface receptor expression in human bronchial epithelial cells. *Virus Research,* 233: 51–59.