

RESEARCH ARTICLE

Effect of the phytochemicals leaf extract of the bacterium Myrrh Commiphora (Guggul) on the pupal parameters of *Bombyx mori* Linn

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Manuscript details:	ABSTRACT
Received: 24.10.2016 Accepted: 24.12.2016 Published : 31.12.2016 Editor: Dr. Arvind Chavhan Cite this article as: Sanjai Kumar Gupta (2016) Effect of the phytochemicals leaf extract of the bacterium Myrrh Commiphora (Guggul) on the pupal parameters of <i>Bombyx mori</i> Linn, <i>International J. of Life Sciences</i>, 4 (4): 609.-614. Copyright: © 2016 Author(s), This is an open access article under the terms of the Creative Commons Attribution-Non-Commercial - No Derivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.	In ancient writings, myrrh (<i>Commiphora myrrha</i> Guggul) is frequently mentioned as a spiritual and therapeutic plant with insecticidal and agricultural advantages. Sericulture depends greatly on nutrition. By enhancing the silkworm's growth, development, health, feed intake, and conversion, it enhances the pupal parameter, which in turn improves the commercial features of silkworm larvae. The monophagous insect <i>Bombyx mori</i> Linn, also known as the silkworm, gets all the necessary elements for its growth and development from the mulberry leaf. Certain plant extracts enhance the nutritional intake, growth, and disease resistance/tolerance of silkworms, ultimately enhancing the cocoon qualities by stimulating feeding. The current study aimed to examine the effects of fortifying Kanva mulberry leaves with botanicals on <i>Bombyx mori</i> pupal development parameters. Since silk is a luxury item that is in high demand, improving the fiber quality is crucial in sericulture. The study looks at how adding ethanolic extracts of Guggul to mulberry leaves affects the development and growth of silkworm pupae parameters, with a focus on the pupation process. Compared to the control group, the supplementation dramatically increased pupal duration, pupal weight, pupal survival, and silk quality. Of the tested concentrations, 5% ethanolic extract had the most significant effect. The phytochemicals from Guggul were added to mulberry leaves to feed silkworms, which produced higher commercial characteristics and pupal life, which act as a natural pupal growth booster in sericulture. This study emphasizes the possibility of employing Guggul supplementation as an environmentally friendly and affordable way to increase silk production and quality, which would ultimately benefit the sericulture industry. Keywords: Myrrh Commiphora plant extract, silk pupal parameters, silk pupal duration parameters, silk survivality of pupae (<i>Bombyx Mori</i> L), Medicinal property of Myrrh (Guggul) leaf.
	INTRODUCTION In Ayurveda, myrrh (<i>Commiphora myrrha</i>) is known as Guggul/ Guggulu, and it is a potent, aromatic resin that has therapeutic and antimicrobial effects akin to those of neem, making it very useful for treating infections in silkworm larvae. Directly contributing to the economic improvement of farmers involved in sericulture is an improved silk production method. Fortifying mulberry leaves with phytochemicals is a potential way to increase the yield and quality of silk in silkworms.

The general health and development of silkworms can be greatly enhanced by fortifying mulberry leaves with extra nutrients like vitamins, proteins, and minerals. Due to its capacity to produce silk, the Mulberry silkworm, *Bombyx mori*, is a valuable insect in the silk industry. These silkworms' nutritional needs are solely satisfied by the mulberry plant, *Morus* spp. The development of silkworm larvae and the resulting silk production are heavily affected by the quality of mulberry leaves. As a contemporary method for raising the economic worth of cocoons, the practice of adding nutrients to improve mulberry leaves and then feeding these improved leaves to silkworms has become increasingly popular. For this reason, phytochemicals plant extract nutrition should provide readily available nutrients to silkworm larvae and be cost-effective throughout the later phases of leaf growth. For this reason, utilizing commercially available myrrh leaf extract samples, this study examines the impact of mulberry feed enhanced with phytochemicals on the development and cocoon quality of *Bombyx mori* L. Myrrh leaf extract is used in the research to fortify mulberry leaves in order to boost the nutritional value and silk production of silkworms. The mulberry leaves that were given to the monophagous silkworm larvae were fortified with a variety of myrrh leaf extract samples. To improve the biological and economic characteristics of silkworms, several methods have been investigated, such as nutritional supplements and bioactive substances (Krishnaswami, 1986). In order to determine the potential of Myrrh (*Commiphora myrrha*) extract as a natural growth enhancer in sericulture, this study examines how it affects the growth, developmental characteristics, and economic traits of *Bombyx mori* L. The study examined the effects of feeding fifth-instar larvae mulberry leaves that had been treated with a 5% ethanolic extract of myrrh (*Commiphora myrrha*) leaves on a number of parameters, including silk quality, shell ratio, shell weight, pupal weight, and cocoon weight. The mulberry silkworm, *Bombyx mori* L., is a domesticated species of economic importance that serves as the foundation of the sericulture industry. It is crucial to increase the development and output of silkworms in order to raise the silk yield and profitability. The use of natural medicines, such as therapeutic herbs, in the treatment of various pathogenic infections has increased in recent years. These goods have been employed to combat the antibiotic resistance and negative side effects of antibiotics. As a result, researchers were compelled to

discover novel antimicrobial compounds that have a long history of usage in ethnotherapy. Plant extracts are less expensive and safer because they contain a large number of phytochemicals. The study of phytoconstituents, which are responsible for the biological effects of therapeutic plants, was stimulated by the production of pharmaceutical products made from medicinal plants (Berg et al. 2015; Vijaya al. 2009 & Das et al. 1950). The leading cause of mortality globally is infectious illnesses. With prolonged usage, many of these illnesses exhibit significant resistance to antibiotics and medications. The antimicrobial capabilities of extracts from some medicinal plants can be used to treat and combat these illnesses. A widely used tree family for the treatment of a variety of bacterial and fungal illnesses, notably Guggul, is the Burseraceae. Nutrition, particularly mulberry leaves, accounts for 35–38% of the growth and silk production of healthy silkworm larvae, which mature into strong pupae, which are necessary for the production of high-quality, heavy cocoons. Proper nutrition and raising practices result in the healthiest possible health, which in turn produces bigger, more resilient cocoons.

MATERIAL AND METHODS

The mulberry silkworm (*Bombyx mori* L.), a Pure Mysore × NB4D2 multivoltine DFLs (disease free egg laying), was raised in plywood trays (23x20x5cm) in laboratory media of mulberry garden in government college under the ideal rearing condition, as described by (Krishnaswami et al. 1986). The silkworm grainage Beharaich, Directorate of Sericulture Uttar Pradesh, provided the eggs. Following conventional sericulture methods the silkworms were raised on mulberry leaves (*Morus alba*) under regulated environmental circumstances. The extract of *Commiphora myrrha* myrrh (Fig.) is well-known for its antioxidant and adaptogenic qualities. In silkworms, tulsi extract increases stress tolerance, especially in challenging environments. This results in continuous silk output and higher cocoon output (Sinha et al. 2009 & Rahamthulla et al. 2007). For thousands of years, they have understood the significance of these fragrant and therapeutic herbs in Ayurvedic medicine. The plant yields important essential oils and fragrance chemicals such as citral, geranial, eugenol, and thymol, which have industrial applications (Jayaprakash Rao, 1999). Myrrh is a medicinal herb collected from my college close to the nursery businesses. The plant leaves were

rinsed with distilled water after being washed with tap water. The extracts were made by grinding them in an electric mixer and mixing them with distilled water at a weight to volume ratio of 1:10, 24–42 hours in a dark environment, followed by 50–600°C heat hot plates for 5–10 minutes. The filtrate, which served as the stock solution, was made by filtering the crushed material solution using a double-layered muslin cloth. 0.5 ml of the stock solution was pipetted and diluted with 5 ml of distilled water to reach a 5% concentration. A fresh dilution was made for use on mulberry leaves and for silkworms to eat, while the stock solution was kept in the refrigerator (Gobena *et al.* 2015). Treatment group: Silkworms fed on mulberry leaves that had been treated with Myrrh *Commiphora myrrha* extract at 5% concentration; control group: Silkworms fed on plant Kanva mulberry leaves. Each treatment had three replicates, and each group was made up of 100 silkworms.

Rearing of silk worms the research utilized third-instar larvae from the (*Bombyx mori* L) race of the Pure Mysore × NB4D2 multivoltine double hybrid silkworm rearing station in silkworm grainage Baharaich, Directorate of Sericulture Uttar Pradesh, India. The Department of Zoology at Government College in Uttar Pradesh, India is where the obtained larvae were nurtured. Fresh, healthy mulberry leaves (Kanva variety) were utilized in the present investigation. The perfect temperature and relative humidity were maintained throughout the experiment at 26±1°C and 80±5% RH, respectively. The guidelines established by (Krishnaswami *et al.* 1986) regarding bed cleaning, spacing, and feeding times were strictly followed. Application method: The use of plant extracts was required and carried out only from the third instar onward. The application timetable is as follows: III instar (first day first feed), IV instar (first and third day first feed), and V instar (every day first feed). The environment for silkworm farming was as follows: - Temperature: 26 ± 1°C, Relative humidity 80±5% RH, and Photoperiod 12±1 hour light and dark cycles. Feeding time of mulberry leaves were washed, air dried, and then sprayed with the proper concentration of Myrrh (*Commiphora myrrha*) extract. The treated leaves were fed to the silkworms four times a day (8:00 AM, 5:00 PM,). The mulberry leaves given to the control group silkworms were not treated. The larval phase maintained the feeding regimes pupal weight, pupal duration, and pupal survival of the mature worm (5th instar larvae when stop feeding),

which was cultivated under optimal conditions such as 26±1°C, 80±5% RH, and 12±1 hours of daily illumination, was placed on a mountage for spinning in order to ascertain the phytochemicals fortification of leaf extract on the pupal and survival of pupae. As a result, the larva transforms into its pupal state and the cocoon is formed. The pupae were kept at a consistent temperature in a BOD incubator. Moths emerged from the pupae after a few days of the pupal phase. Determining how long the pupa lasts are the period between the third day of spinning (pupae formation) and the appearance of the moth was taken into account. To this aim, 75 cocoons, each containing a pupa (three batches of 25 cocoons apiece), were collected for study. Each experiment was repeated three times and calculates the weight of the pupa. The weight of 30 healthy pupae three groups of ten was noted for each replication. On the seventh day after the creation of pupae, the pupal weight was measured. For documenting the survival of pupae are 75 pupae were noticed, comprising three batches of 25 healthy pupae each. The survival rate of pupae was determined by counting the number of pupae that turned into moths, as follows:

$$\text{Pupae survival} = \frac{\text{Number of moths emerged}}{\text{Number of pupae used for observation}} \times 100$$

The data collected was subjected to statistical analysis using a one-way ANOVA. Statistical Analysis: Using Microsoft Excel, the data were analyzed statistically and presented as means SD values.

RESULT AND DISCUSSION

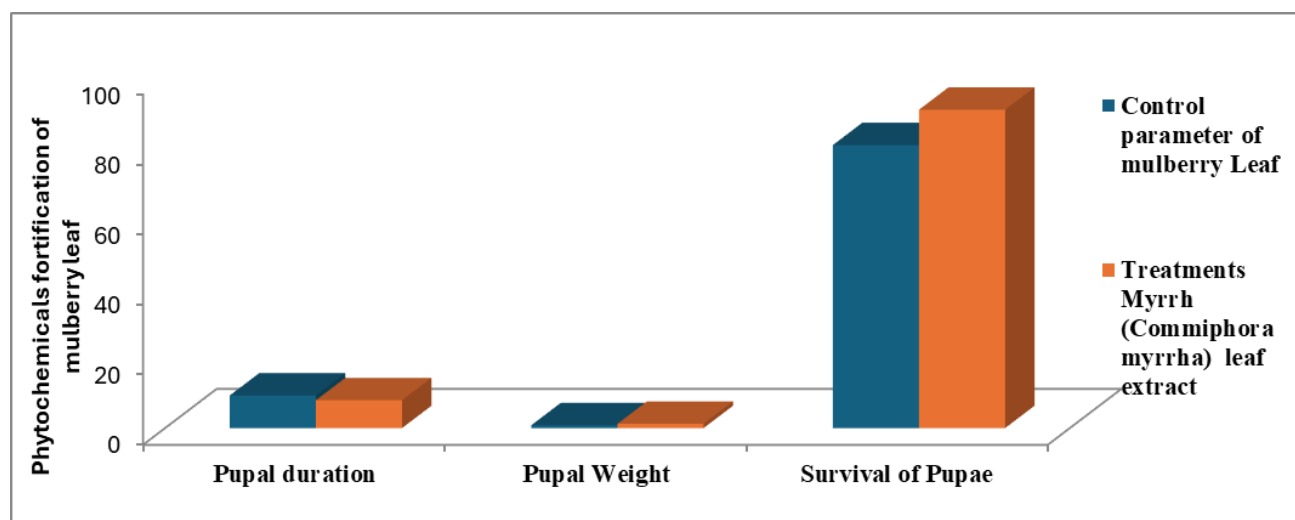
As shown in (Table Fig) the phytochemicals ethanol extracts of Myrrh (*Commiphora myrrha*) and mulberry leaves had an impact on the various pupal parameters of the silkworm, such as pupal length, weight, and survival. The over modifications are examined in relation to the silkworm's pupal features such as pupal duration, pupal weight, and pupal survival. Mulberry leaves and myrrh (*Commiphora myrrha*): Table displays the impact of phytochemicals leaf extract from Myrrh (*Commiphora myrrha*) on the characteristics of silkworm pupae. The pupal nature of the control group increased significantly, with the 5% phytochemicals *Commiphora myrrha* extract showing a larger increase. The average pupal weight was greater (1.22 g) when *Commiphora myrrha* plant extracts were utilized, according to the study, than when the control group was used (0.81 g).

Table: 1- Effect of Supplementary Myrrh *Commiphora Guggle* phytochemicals ethanolic plant extract on different stages pupal parameters of silkworm *Bombyx mori* L.

Sr. No.	Phytochemicals fortification groups	Pupal duration (days)	Pupal Weight (gm)	Survival of Pupae (%)
1-	Control parameter of mulberry tree leaves	9.30 ±0.130	0.81 ±0.016	80.73 ±0.876
2-	Phytochemicals treatments Myrrh (<i>Commiphora myrrha</i>) leaf extract	7.951 ±0.232	1.22 ±0.028	90.82 ±0.542

*p<0.01 F-ratio= n1=4 n2=10 ANOVA F-ratio=36.84

Each value represents mean ±S.D. of three replicates.



Effect of Supplementary Myrrh *Commiphora myrrha* phytochemicals ethanolic plant extract on different stages pupal parameters of Silkworm *Bombyx mori* L.

This implies that the Myrrh extract (*Commiphora myrrha*) can alter the pupal characteristics that are included in the pupal parameters list. Compared to phytochemicals treated mulberry leaves (7.951 days), phytochemicals-treated fifth instar larvae displayed a noticeable improvement in pupal weight and pupal survival as well as a reduction in the pupal duration as control (9.30 days). The average filament length is used to categorize the silkworm species. The pupae survival percentages are (80.73%) when employing control parameters, and the phytochemicals leaf extract raises the percentage of pupae survival circumstances (90.82%). In this study, phytochemicals enrichment using plant extracts dramatically extended pupal life when compared to controls. Phytochemicals Ethanolic plant extract Guggul, which regulates the bacterium pest, cures pupal parameters. Compared to the control groups the maximum output of pupal parameters and cocoon production results are in high cocoon length which increase filament length. This can

improve pupal development and cocoon formation, which are beneficial for sericulturist rearers to increase the cocoon productivity in the next generation of multivoltine mulberry silkworm larvae.

The positive effects of ethanolic *Commiphora myrrha* phytochemicals extract on silkworm economic growth and development are seen in the increased pupal length, pupal weight, and survival of pupae, especially in imported hybrids. These findings show that, as an alternative to synthetic chemicals, phytochemicals plant extracts are a feasible and ecologically sound way to boost silkworm output (Etaber and Kaliwar 2004 & Hajam *et al.* 2014). By lowering the inoculums levels of pathogens, the bio soft descent method using silkworm pupal residue may provide a non-chemical way to combat plant diseases. Such a management would help in preventing the pollution and also health risks. The correct use of silkworm pupal residue as a byproduct produced in large quantities by silk reeling

factories, which would otherwise be wasted, is made possible by the bio soft descent method of silkworm pupal residue treatment for disease control. Local hybrid positive reactions that were seen indicated the possibility of maximizing silkworm raising in particular agricultural settings (Radha NV *et al.* 1988 & Quiyyum MH 1990). The current study's findings are consistent with those of (Khan MA *et al.* 2010 & Jiang Li Peng *et al.* 2016), which revealed that 5% concentrations of myrrh (*Commiphora myrrha*) phytochemicals increased the average filament length by 1221 m, compared to 1071 m in the control group. One research discovered that adding 20% Parthenium leaf extract produced the longest silk filament, which measured 873 meters. According to this discovery, the seed extract of *Trichosanthes cucumerina* produced the highest cocoon weight, shell weight, silk ratio, and filament length (Khaliknazarov U *et al.* 2012 & Chiranjib B *et al.* 2011). According to (Akbar MA *et al.* 2009 and Mohmoud SM *et al.* 2016), the highest cocoon weights were produced by larvae fed mulberry leaves with 3.2% basil leaf extracts, while 3.1% black cumin seed extracts yielded significantly higher cocoon shell weights. Furthermore, the Cocoons had greater silk ratios across all tested quantities of black cumin seed extract. Similar outcomes were observed for the shell ratio in (Chen JJ *et al.* 2010 & Alam MK *et al.* 2014) (Vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% + methionine 0.5%). T5 had the greatest mean cocoon shell ratio at 24.59 %. (Sujatha *et al.* 2015) The 3% (T3) Myrrh (*Commiphora myrrha*) enhanced, resulting in a notable increase in economic features when compared to the control. After leaf treatment with extracts from *T. terrestris*, *B. diffusa*, and *P. niruri*, there was a clear correlation between silkworm development and silk manufacture. The plant extracts promoted silkworm development because they had an active biological component that facilitated the silkworms' digestion of nutrients (Chaves N *et al.* 2015 & Arora DS *et al.* 2007) Plant extracts from *Sida acuta* were added to methanol for supplementation, which had an impact on silk and larval traits. The growth and development of *Bombyx mori* is aided by adequate diet. The addition of 5% tulsi plant extract enhances larval and silk production. The end outcome of the trial group on the silkworm was an increase in the length of the silk, the weight of the larvae, and the length of the silk. In order to improve the growth and cocoon characteristics of silkworms, the *B. mori* the *Sida acuta* plant extract was tested against 5th instar larvae of silkworms by (Atef

NM *et al.* 2015 & Chao PY *et al.* 2014). Numerous research have demonstrated that varying concentrations of plant extract and their natural composition, which includes Ascorbic acid, folic acid, thiamin, vitamins, hormones, Aloe tonic, and other ingredients, enhance the characteristics of larvae and cocoons. Numerous attempts have been undertaken to raise the quantity and quality of silk manufacturing, including nutrient-boosting leaves, antibiotic spraying, vitamins, hormones, plant products, and extracts. Phytochemicals have the potential to influence insect survival and function, and plants have the highest concentration of organic molecules on the plant (Ayyappadhas R *et al.* 2012 & Cantrell L Dayan *et al.* 2012). The significance of this plant in treating certain fungal and bacterial infections was highlighted by these results. These enhancements ultimately result in a better pupal existence, as seen by metrics such as pupal length, pupal weight, pupal survival, and economic factors of the cocoon in nutrient treated silkworms.

CONCLUSION

One of the key species in the sericulture industry is the *Bombyx mori*, the multivoltine mulberry silkworm. It is in great demand in the agricultural market because it is made of yellow-colored silk naturally. The key factors in silkworm rearing are good agro-climatic and environmental conditions, as well as healthy seeds and host plants. In successful silkworm rearing, one healthy host plant is extremely crucial. Due to significant pest infestation of host plants, the current study found that several insect pests are the primary cause of mulberry silkworm culture. Defoliators are the most damaging insect pests of the mulberry tree. For the protection of mulberry silkworm food plants, scientific approaches to insect pest control are therefore essential. According to the research, a 5% Myrrh (*Commiphora myrrha* Guggul ethanolic) plant extract greatly enhanced the beneficial qualities of silkworms as well as all pupal characteristics. The results emphasize the possibility of employing fortified mulberry leaves as a calculated nutritional intervention to improve silkworm rearing, which will eventually help sericulturists by enhancing the output and quality of raw silk. The environmentally friendly and affordable approach to addressing nutritional deficits in silkworm diets, leading to improved economic results. Future research should concentrate on isolating the active components in these extracts

and improving their application techniques in order to increase the efficiency and scalability of this fortification strategy in sericulture. The possibility that the ethanolic plant extract of Myrrh (*Commiphora myrrha* Guggul) might be used as a natural growth promoter in sericulture is highlighted by these results. Consequently, plant extract containing these phytochemicals holds promise as a useful suggestion for rearers.

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REFERENCES

- Akbar MA Ahamed R Alam KD Ali MS (2009) In vitro cytotoxic properties of ethanolic extracts of various parts of *S. mahagoni*. *Eur J Sci Res* 32(4):541-544.
- Alam MK Mansur FJ Karim MM Haque MA (2014) Antimicrobial activity of *S. mahagoni* (Seed) against various pathogenic microbes. *Indo Am J Pharm Res* 4(05):2362-2366.
- Arora DS Kaur GJ (2007) Antibacterial activity of some Indian medicinal plants. *J Nat Med* 61:313-317.
- Atef NM Shanab SM Negm SI Abbas YA (2015) Evaluation of antimicrobial activity of some plant extracts against antibiotic susceptible and resistant bacterial strains causing wound infection. *Bull Natl Res Cent* 43:144-146.
- Ayyappadhas R Jestin C Kenneth N Dayana N Dhanalekshmi UM (2012) Pre-liminary studies on antimicrobial activity of *S. macrophylla* leaf extract. *Int J Pharm Sci Rev Res* 16:1-4.
- Barge SB and AB Pardeshi (2015) Influence of dietary supplementation with of *Sida acuta* plat extract on the mulberry silkworm, *B. mori* L. *International Journal of Zoology Studies* 3(2) 199-202.
- Cantrell L Dayan E Duke O (2012). Natural products as sources for new pesticides. *J Nat Prod.* 75(6):1231-1242.
- Chao PY Lin SY Lin KH, Liu YF Hsu JI Yang CM Lai JY (2014) Antioxidant activity in extracts of indigenous Taiwanese vegetables. *Nutrients* 6(5):2115-2130.
- Chaves N Santiago A Alías JC (2015) Quantification of the antioxidant activity of plant extracts: Analysis of sensitivity and hierarchization based on the method used. *Antioxidants* (basel 9(1):76-80.
- Chen JJ Huang SS Liao CH Wei DC Sung PJ Wang TC Cheng MJ (2010) A new phragmalin-type limonoid and anti-inflammatory constituents from the fruits of *S. macrophylla*. *Food Chem.* 120:379-384.
- Chiranjib B Laxmaiah Ch Srikanth V Gouda TS Kumar CS, Debnath S (2011) Antimicrobial activity of *S. mahagoni* L. (leaf) against various human pathogenic microbes. *Indo Am J Pharm Res* 257-261.
- Das GMR (1950) Diseases of silkworm monograph on cottage industries. No1, Govt. India Press,
- Etaberi K Kaliwal B (2004) Matindoost L. Supplementation of mulberry leaves in sericulture, theoretical and applied aspects. *Int. J Indust Entomol* 9: 14-28
- Gobena W Sori and R N Bhaskar (2015) Fortification of mulberry leaves with medicinal botanical plant extracts effect on silkworm, *Bombyx mori* L.(PM×CSR2)(Lepidoptera: Bombycidae) larval growth and cocoon traits 199-206.
- Hajam O Ahmad (2014) Influence of plant extract fortified mulberry leaf on some reeling parameters of silkworm, *Bombyx mori* L 124-127.
- Jayaprakash rao (1999) "Effect of few Botanicals and iodine compounds on the economic traits of eri silk worm M. Sc (Sericulture) Thesis, University of Agriculture Science, Dharwada 199-209.
- Jiang Li Peng L Cao Yu Yao Thakur K Hu F Tang S & Wei ZhaoJun (2016) Effect of Dietary Selenium Supplementation on Growth and Reproduction of Silkworm *Bombyx mori* L Biological Trace Element Research 193: 271-281 .
- Khaliknazarov U Akbarov D Tursunov A Gafforov S & Abdunabiev D (2012) Existing problems of drying cocoon and making chrysalis feeble, and their solutions. IOP Conference Series: Earth and Environmental Science, 939(1).
- Khan MA Akram W Ashfaq M Khan HA Kim YK (2010) Effects of optimum doses of nitrogen, phosphorus, potassium and calcium on silkworm, *Bombyx mori* L., growth and yield. *Entomol Res* 40(6): 285-289.
- Krishnaswami S (1986) Sericulture Manual II: Silkworm Rearing. FAO Agricultural Services Bulletin. 13.
- Mahmoud SM (2016) Silk agriculture and industry Noor publishing, GmbH Germany 1288-291.
- Qaiyyum MH Bari MA (1990) Foliar nutrition in mulberry. *Indian Silk* 28(1): 26.
- Radha NV Nagarajan P Jayaraj S (1988) Mineral deficiency in mulberry plants, *Morus alba* L. and its effect on economic characters of silk worm, *Bombyx mori* L. *Madras Agric J* 75(11): 384-390.
- Rahmathulla VK Priyabrata D Ramesh M Rajan RK (2007) Growth rate pattern and economic traits of silkworm, *Bombyx mori* L under the influence of folic acid administration. *J Appl Sci Environ Mgmt* 11(4): 81-84.
- Sinha USP Prasad J Das S Ojha NG Vijay Prakash NB (2009) Effect of secondary nutrients on leaf yield and biochemical constituents of *Terminalia tomentosa*. *Int j of Ind Entomol* 18(2): 135-138.
- Vijaya D Yeledhalli NA Ravi MV Nagangoud A Nagalika VP (2009) Effect of fertilizer levels and foliar nutrition on M-5 mulberry leaf nutrient content, quality and cocoon production. *Karnataka J Agric Sci* 22(5):1006-1012.