Chapter 6

STUDIES ON ANTIMICROBIAL ACTIVITY AND ENHANCEMENT OF SILK PRODUCTIVITY OF ERI SILKWORM OF SOME SELECTED METAL COMPLEXES

The work embodied in this chapter is divided into two parts (A & B). **Part A** describes the antimicrobial activity of some selected cadmium complexes towards some specific strains and **Part B** deals with the effect of some copper and zinc complexes on the production of silk by eri silkworm *(Samia cynthia ricini* Boisd *)*

PART A: Antimicrobial study

6.A.1 Introduction

Heavy metals have continued to draw widespread attention owing to their release into the environment, extended persistence, and toxicity to a wide variety of organisms including

different microorganism. Though cadmium is not a biological essential element but biological interest in cadmium is based on the fact that its presence in living system adversely affects the metabolism, giving rise to acute or chronic toxicity. The Cd^{2+} ion has long biological half life (about 30 years in mammals) and slow excretion of Cd^{2+} contribute to its accumulation in living cells, causing damage in the liver, kidneys, lungs and nervous system. Thus cadmium has been classified among the toxic elements **[1,2].** The divalent metal ion directly inhibits succinate dehydrogenae and the respiratory chain in isolated liver mitochondria **[3]** and cause renal disturbances, lung insufficiency, bone lesions, cancer and hypertension in humans **[4]**. Cadmium has strong affinity to form complexes with biologically relevant ligands disrupting the biological function of these ligands. The stability of cadmium complexes varies depending on the type of ligands. Cadmium, a soft Lewis acid, prefers easily oxidizable soft ligands as per SHAB principle ,not dismissing its affinity for other ligands, above all when the chelate effect comes into play[**5]**. Their mobilisation depend on the binding of ligands via nitrogen atoms.

Antimicrobial resistance is fast becoming a global concern with rapid increase in multidrug-resistant bacteria and fungi [**6**]. This has mandated continued search for new antimicrobial compounds, including coordination complexes of biologically important molecules [**7**]. The increased lipophilic character of these coordination compounds, with the resultant enhanced ability to permeate accounts for their improved activity over their parent ligands [**8,9**]. Chelation, which has been reported to reduce the polarity of the metal ion by partial sharing of its positive charge with the donor group of ligands, also supports this theory **[10].**

Cadmium is an important environmental pollutant and a potent toxicant to bacteria, algae, and fungi. Mechanisms of cadmium toxicity and resistance are variable, depending on the organism. The routes by which cadmium finds entry into the environment are industrial sources, burning of fossil fuels and recycling electronic waste - the molecular mechanisms behind its toxicity in cells, as to how it leads to oxidative stress are not understood A wide range of cadmium concentrations have been used for studies involving resistance in organisms. No concentration has been specified till date that is applicable to all species studied under standardized conditions. The metal thus exerts its toxic effect(s) over a wide range of concentrations. In most cases, algae and cyanobacteria are the most sensitive organisms, whereas bacteria and fungi appear to be more resistant. In some bacteria, plasmid-encoded resistance can lead to reduced Cd^{2+} uptake. However, some gram-negative bacteria without plasmids are just as resistant to Cd as are bacteria containing plasmids encoding for Cd resistance. Mechanism of cadmium uptake and resistance in cyanobacteria and algae remain largely undeciphered. Cadmium is toxic to these organisms, severely inhibiting several physiological processes - growth, photosynthesis, and nitrogen fixation at concentrations less than 2ppm, and often in the ppb range. Cadmium also causes pronounced morphological aberrations in these organisms, which are probably related to deleterious effects on cell division. Cadmium may affect protein synthesis and cellular organelles such as mitochondria and chloroplasts. Cadmium is accumulated internally in algae as a result of a two-phase uptake process. The first phase involves a rapid physicochemical adsorption of Cd(II) onto cell wall binding sites, which are probably proteins and (or) polysaccharides. This is followed by a lag period and then a slow, steady intracellular uptake. This latter phase is energy dependent and may involve transport

systems used to accumulate other divalent cations, such as Mn^{2+} and Ca^{2+} **[11-15].** In this context, it is pertinent to mention herein that though quite a large number of cadmium metal complexes have been screened for their antimicrobial activity – most of these compounds are often insoluble, contain macrocyclic Schiff base ligands or carbamate ligands [**7**]. Such study involving complexes that are water soluble and biocompatible containing biogenic ligands are scanty **[16-18].** Metal complexes of some amino acid derived ligands have been shown to exhibit *in vitro* antimicrobial and antifungal activity [**10**]. Imidazole derived transition metal complexes have also been investigated for their antimicrobial activity [**19**]. Compounds with aminoacids as ligand have shown promising antimicrobial activity [**17, 18, 20**]. Cadmium(II) complexes of glycine and phenylalanine have shown antimicrobial activity against *Bacillus subtilis, Staphylococcus aureus,* methicillin resistant *Staphylococcus aureus* (MRSA), *Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris and Candida albicans* [**21**] .

In light of aforementioned discussion it was considered worthwhile to investigate some of the newly synthesised mixed ligand cadmium fluoro complexes (**Chapter 5**) for antimicrobial activity towards selected bacterial strains

6.A.2 Experimental

Antimicrobial activity against two gram positive strains, *Staphylococcus aureus* [A] and *Bacillus subtilis*[B], and two gram negative strains, *Klebsiella pneumonia* [C] and *Escherichi coli*[D] were studied. The experiment was carried out following Kirby-Bauer disc diffusion technique and the extent of antimicrobial activity was evaluated by measuring the diameter of zone of inhibition as per standard protocol **[22]**. To obtain bacteria in the mid-logarithmic phase 100µl of nutrient of an overnight culture made in

nutrient broth was added to 10 ml of nutrient broth and incubated for 5 hours at 37 °C with orbital shaking for each strain. The strains were separately plated on nutrient agar media by pour plate method. The test samples were then dissolved in water at a concentration of 50µg/ml and adsorbed on the sterile paper discs. The discs were placed on the agar medium and the plates were incubated at 37 °C for 24 hours. The resulting inhibition zones on the plates were measured after 24 hours. The tests were carried out in triplicate and the results are expressed as mean. Two standards *viz*. ceftazidine and azithromycin for the gram-positive and gentamicin and impipenem for the gram-negative strains were chosen. To study their antimicrobial properties, four water soluble cadmium complexes, $[CdF_2(Hser)(H_2O)]$**(20)** $[CdF_2(Hcys)H_2O)]$**(22)**, $[CdF_2(im)(H_2O)]$**(23)** and $[CdF_2(bim)(H_2O)]$ (**25**), were selected.

Results and Discussion

Four water soluble cadmium complexes, $[CdF_2(Hser)(H_2O)]$ **(20),** $[CdF_2(Hcys)H_2O)]$ **(22),** $[CdF_2(im)(H_2O)]$ **(23)** and $[CdF_2(bim)(H_2O)]$ (**25**) were selected for the antimicrobial activity study. **Fig.6.1 and Table 6.1** depicts the diameter of inhibition zones on the petri dishes. **Fig. 6.2** portrays 3D bar diagrams representing the extent of antimicrobial activity of the compounds and standards. Interestingly the compound, $[CdF_2(Hcys)(H_2O)]$ **(22)** was found to be ineffective against all the selected bacterial strains while none of the test compounds had any inhibitory effect towards the *Klebsiella pneumonia* strain. The compounds $[CdF_2(Hser)(H_2O)]$ **(20),** $[CdF_2(Im)(H_2O)]$ **(23)** and $[CdF_2(bim)(H_2O)]$ **(25)** were found to be quite effective towards the three strains, *Staphylococcus aureus* [A] and *Bacillus subtilis* [B], and *Escherichia coli* [D]. The inhibitory effect was found to be more pronounced for the *Escherichia coli strain.* The reason for the inactivity of the

$[CdF_2(Hcys)H_2O)]$**(22)** compound is not apparently understood. However, it may be worthwhile to note that the coligand cystiene in **(22),** unlike the rest complexes, contain a thiol function.

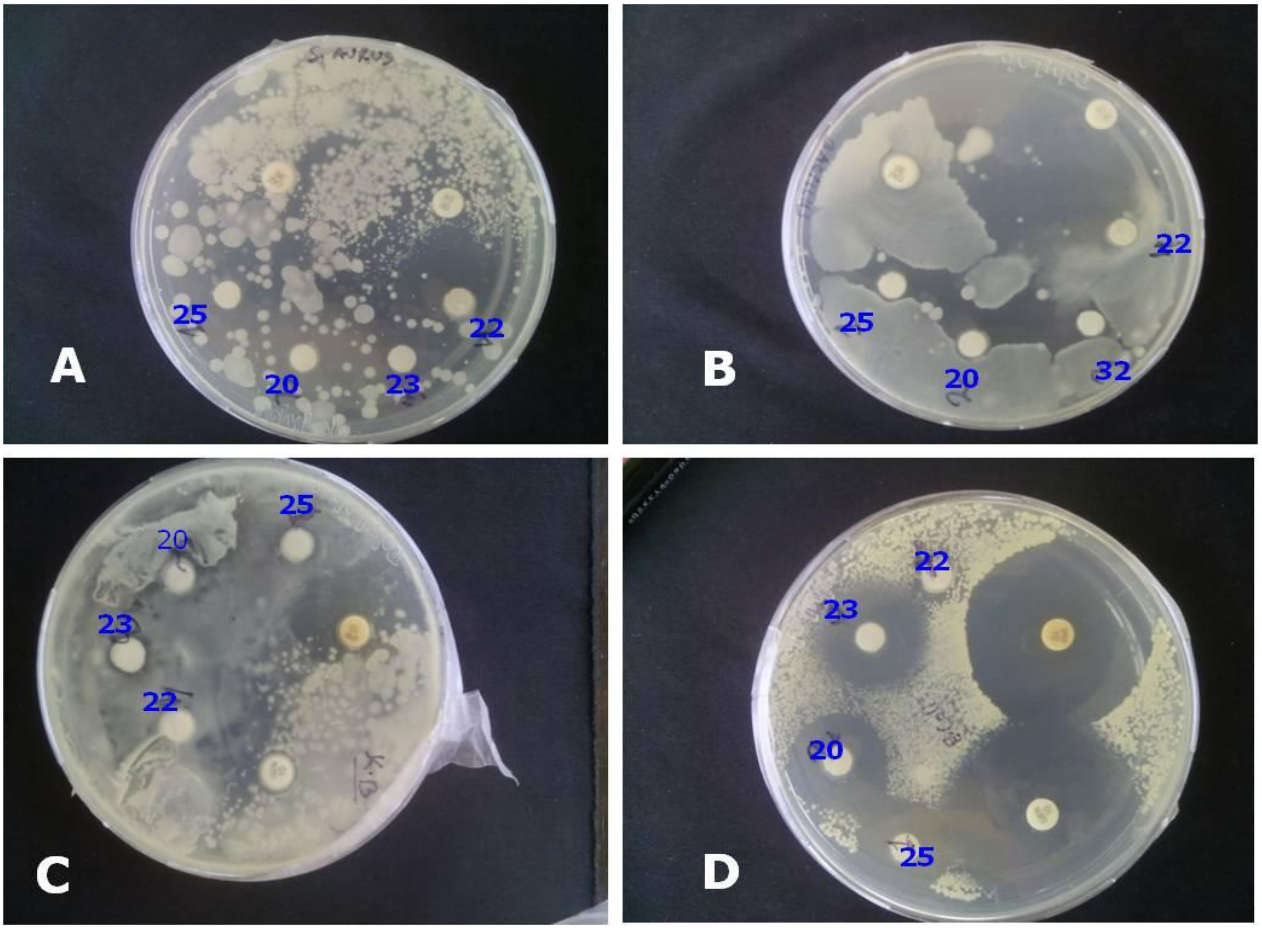


Fig. 6.1 Depiction of the diameter of inhibition zones on the petri dishes : $[CdF_2(bim)(H_2O)]$ **(25)**,$[CdF_2(Hser)(H_2O)]$ **(20)** ,$[CdF_2(im)(H_2O)]$ **(23)** and $[CdF_2(Hcys)H_2O)]$ **(22)** on four bacterial strains: *Staphylococcus aureus* [A], *Bacillus subtilis* [B], *Klebsiella pneumonia* [C] and *Escherichia coli* [D]

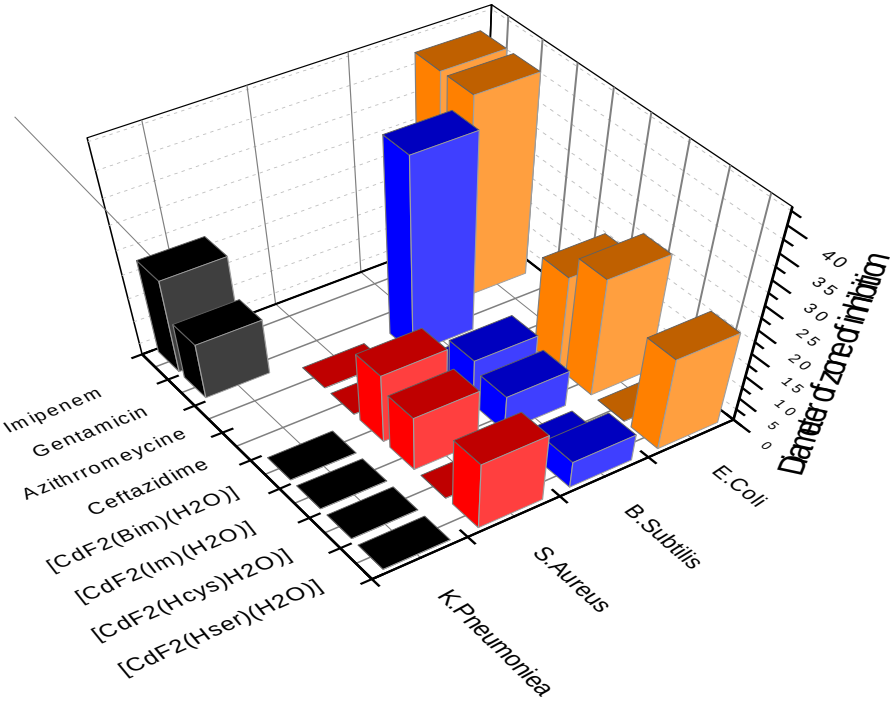


Fig. 6.2 : 3D bar diagrams representing the extent of antimicrobial activity of the compounds and standards

As already noted, literature reports dealing with cadmium(II) complexes are mostly related to macrocyclic or multidentate Schiff base ligands [**23-26**]. The Schiff base itself and very often the corresponding complexes are not water soluble and thus likely to be toxic and not effective under normal physiological conditions. The newly synthesized compounds reported in **Chapter 5** used as test compounds in the present experiment incorporates simple biogenic coligands and their solubility in aqueous medium makes an ideal case for antimicrobial compounds with least toxic side effects.

Table 6.1 : Diameter of zone of inhibition of selected Cd-compounds **(20,22,23** and **25)**

Compound	**Diameter of zone of inhibition (mm)**			
	S.aureus	***B. subtilis***	***K. pneumoniea***	***E. coli***
$[CdF_2(Hser)(H_2O)]$ **(20)**	15.0	6.0	0.0	20.0
$[CdF_2(Hcys)(H_2O)]$ **(22)**	0.0	0.0	0.0	0.0
$[CdF_2(Im)(H_2O)]$ **(23)**	12.0	8.0	0.0	25.0
$[CdF_2(bim)(H_2O)]$ **(25)**	15.0	10.0	0.0	20.0
Ceftazidine **(CAZ)**	0.0	0.0	-	-
Azithrromycine **(AT)**	0.0	40.0	-	-
Gentamicine **(GEN)**	-	-	12.0	40.0
Imipenem **(IPM)**	-	-	20.0	40.0

PART B : Metal compound(s) induced enhanced silk productivity

6.B.1 Introduction

Silk worms are considered as the unique gift of nature. These insects have been exploited as a source of natural protein fiber, some of which can be woven into textiles. India is home to a total of 47 species of silkworms of which 24 reported from North East region. However, only four species of sericigenous insects - muga, eri, tussar and mulberry are cultured. Among these eri and muga silkworms are predominantly cultured in North East part of India. Worldwide there are 19 varieties of eri (genus:Samia) of which only three species are reported from India with two from NE region. These are *Samia canningi*, a wild species and *Samia ricini*, a domesticated species. India is unique in having four types of silkworms - *Bombyx mori; Antheraea mylitta*; *Samia cynthia* and *Antheraea assama*. The *Antheraea assama*, in particular, is famous for the golden silk, which has found mention in literature as early as 1662 B.C. It is semi-domesticated. The golden silk produced by it is a special feature of Assamese culture and tradition that has caught worldwide attention. Another variety, *Samia cynthia ricini* commonly known as eri silkworm produces a silk fiber which is an integral part of indigenous textiles of North East India especially among the socio-economically backward and tribal people. The North East region contributes roughly over 80% of total Indian sericultural production. The market share of Indian silk exports in the global silk trade is 4-5% only which is not significant considering that India is the second largest producer of raw silk. This is because India has a large domestic market for silk goods and about 85% of silk goods produced are sold in the domestic market. However, India exports approximately 15% of its output of

all types of silk goods (including value-added items). The export of silk products has been showing a steady growth and a rapid increase in the export earnings has been registered during the last decade [**27**].

Eri-culture is also quite popular in other countries like Tibet, Myanmar, Japan, China etc. Most of the producer of eri silk worm also uses the insect as food which contains high amount of protein. The larval content of eri-silkworm is rich in protein. All the verities of silkworm has been cultured in almost the same traditional ways. Silk worm nutrition is an important aspect in sericulture, as the growth of silkworm and development of silk production prominently depends upon it. Nutrition can include minerals, amino acids, proteins, vitamins and sugars. Although silkworm is cultured quite extensively in different parts of Asia, research on growth enhancement and silk productivity have not received due attention. Kobayashi [**28**] undertook a pioneering investigation on the effects of juvenile hormone(JH) inducing quantitative increase in the silk production by *B. Mori* opening a new horizon for silk production enhancement. A number of papers dealing with this aspect appeared as a follow-up [**29-35**]. All such growth of silkworm in terms of prolongation of larval life is associated with increase in larval weight and increase the production of silk. Recently studies on influence of metal based compounds like salts of zinc, nickel etc has revealed promising results in the production of silk by silkworm [**36-41**]. Zinc plays an important role in augmenting the growth and antioxidant protection of the larva of *Antheraea myli tta* wherein it is implicated in improvement of the larval fitness, quality and quantity of silk production of *Antheraea mylitta* [**42**]. This particular species yields a variety of silk commonly called tussar. Zinc is an essential element for the growth, hormonal balance and wound healing [**43, 44**]. It is known that zinc helps in peptisation

and thus enhance the production of silk by the insect which is formed from polymerisation of different amino acids. Zinc-salts are known to modulate the production of cocoon and silk of Bombyx mori [**45**]. A number of studies on zinc supplementation effect on silk production parameters have been documented [**37-41, 46**]. Besides minerals, silkworms do not have enzymes required to synthesize all the amino acids. The six amino acids which silkworm can produce are proline, alanine, glycine, serine, tyrosine and cystine. Other essential amino acids - arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (group 1) and aspartic acid and glutamic acid (group 2) needed for synthesis of silk are procured through diet are. These amino acids are required for the growth of the larva [**46**, **47**]. The posterior division of the silk gland of the silkworm, conventionally called posterior silk gland(PSG), synthesize exclusively a single exportable protein fibroin, an important silk protein with a simple amino acid composition - rich in glycine, alanine, serine, and tyrosine. Fibroin is synthesized very rapidly in the posterior silk gland during the fifth instars. A number of papers have addressed this aspect of amino acid supplementation effect on silk production by silkworms [**47-50**]. Effect of leucine and valine supplementation in eri silkworm with regards to cocoon characters have been studied recently [**51]**. Enrichment of the leaves by dietary supplement is one of the strategy by which silk production can be enhanced. Similar studies involving aspargine and alanine on *B. mori* has been carried out[**48**]. The larval content of eri silkworm is full of protein. Increase in silk gland weight leads to higher shell weight i.e. the silk thread production. The quality of cocoon of eri silkworm is a polyphagous, multivoltine worm whose primary host is castor. They also fed on kesseru, bor kesseru, gamari, payam, topioca. An assessment of castor, kesseru and gamari leaves

as dietary supplement for eri silkworm has been made as a part of the present research program. Having accomplished successful synthesis of water soluble mixed ligand zinc-amino acid and copper-amino acid complexes, it was deemed fit to assess the effect of their supplementation through diet for enhancement of silk production by the eri silkworm, *Samia cynthia ricini*. Accordingly this study formed a part of the biological investigation of the newly prepared complexes.

6.B.2 Experimental :

6.B.2.1 Disease free layings (DFL) collection :

Initially the DFLs were collected from Central Silk Board, Diphu , Karbi Anglong district, Assam. Subsequently the eggs of 2^{nd} and 3^{rd} rearing were used for further experimentation.

6.2.2 Methods

Rearing procedure :

Rearing was done indoor at the Lumding College Campus. The larvae were fed on fresh castor leaves. To avoid pesi infestation and predator attack, the larva were reared inside well ventilated rearing cages made of bamboo. During the period of moulting, feeding was discontinued and the larvae were kept untouched for undisturbed moulting. Larvae were properly cleaned before feeding. Tender, Semi-mature and mature leaves were fed during 1^{st} and 2^{nd}; $3^{rd}$and 4^{th} and 5^{th} instars, respectively. Completely dry and wet leaves were avoided. Newly emerged larvae (50 nos.) were separated in three different types of plant leaves (castor, kesseru, gamari) during the whole period of larval instars. Young leaves were fed upto the 3^{rd} instars while mature leaves were given thereafter. Comparative larval weights for the three leave fed cases were recorded in the first day of each instars

(**Table 6.1**). Cocoon weight, shell weight and shell ratio percentage (**SR%**) were also recorded (**Table 6.2**).

Treatment:

To the baby larva normal feeding of castor leave as mentioned earlier were applied up to their 5^{th} instars level. Just at the beginning of their 5^{th} instars, healthy larva of equal weight were separated in to four groups(Gr-I, Gr-II, Gr-III and Gr-IV), each containing 12 number.

Four stock solution containing 1% aqueous solution of the selected compound, $[Cu(val)_2(H_2O)]$ **(10)**, $[ZnF_2(Hser)(H_2O)].4H_2O$ **(13)** and $NH_4[ZnF(ser)_2(H_2O)].5H_2O$ **(14)** were prepared in distilled water and labeled. In the Gr-I insects, 5µl of $[Cu(val)_2(H_2O)]$ (10) per each insect, was applied on the castor leave and allowed to eat. It was carefully monitored so that one larva can have 5µl of the compound along with the castor leave. Similarly, Gr-II insects were treated with the compound $[ZnF_2(Hser)(H_2O)].4H_2O$ **(13)** and Gr-III insects were treated with the compound $NH_4[ZnF(ser)_2(H_2O)].5H_2O$ **(14)** Gr-IV insects were taken as control and allowed to feed on castor leave only without any supplementation. Larval weight, length and weight of PSG were monitored. The weight of cocoon, pupa, shell, silk and the moth and SR% (shell : cocoon weight ratio) were also recorded. The body weight was calculated from disease free, well-fed larvae and an average of 10 larvae at a time was taken. Underfed and diseased larvae were not considered for taking body weight. The body weight of larva was recorded during the 5^{th} instars from day 1 to day 6 during their growth phase using a digital balance and expressed in g/larva. The weight of extracted PSGs were taken at the end of fifth instars separately. The wet weight of PSG was taken for both control and treated insect and expressed in g/pair.

6.B.3 Results and Discussion

The eri silkworm mainly feeds on castor plant(*Ricinus communis*). The other food plants are kesseru (*Heteropanax fragrans seem*) and gamari (*Gmelina arborea*) etc. Investigations were made to assess the three food plant leaves of castor, kesseru and gamari towards improving the economic parameters of eri-silkworm. The results (**Table 6.1** and **Table 6.2**) indicate that castor leave fed eri-silkworm revealed highest weight in all the instars followed by those of kesseru leave and then gamari leave.

Table 6.1 : Weight of larvae of different leave fed in different instars

Food Plant	1^{st} Instar	2^{nd} Instar	3^{rd} Instar	4^{th} Instar	5^{th} Instar before moulting
Castor	0.025	0.25	0.30	0.66	6.01
Kesseru	0.03	0.12	0.19	0.50	5.10
Gamari	0.02	0.30	0.04	0.11	3.55

*As the 1^{st} instar larvae were very small, the weight recorded is average of 10 larvae after 1 day

Based on the outcome of the feeding experiment just discussed castor leaves were selected for the next phase of the study involving the newly synthesised complexes of copper and zinc as dietary supplements. Aqueous solution of the selected compounds were applied on the castor leaves during feeding schedule. In the said experiment, it was observed that the compound $[Cu(val)_2(H_2O)]$ **(10)**, and $[ZnF_2(Hser)(H_2O)].4H_2O$ **(13)** had no positive impact on the production of silk. In fact, the compound $[Cu(val)_2(H_2O)]$ **(10)**, decreases the mortality rate of the larva. But the compound $NH_4[ZnF(ser)_2(H_2O)].5H_2O$ **(14)** (note: the composition is different from the compound **13**) shows a tremendous busting effect on the production of silk.

Table 6.2: Shell weight, cocoon weight and SR% in three different rearing

Castor Fed					Kesseru Fed					Gamari Fed				
Cocoon Wt. in g		Shell wt. in g		SR%	Cocoon Wt. in g		Shell wt. in g		SR%	Cocoon Wt. in g		Shell wt. in g		SR%
	Average		Average			Average		Average			Average		Average	
2.40		0.30			1.98		0.18			0.65		0.08		
2.59		0.38			1.49		0.10			0.90		0.09		
2.62	2.48	0.44	0.30	15.32	1.84	1.82	0.11	0.13	7.14	0.82	0.78	0.07	0.08	10.26
2.20		0.30			1.92		0.16			0.62		0.05		
2.60		0.46			1.89		0.13			0.92		0.10		

On application of this compound, production of silk increases manifold. It is observed that during June-July session the mortality rate of the 5th instars larva increases. This may be due to higher temperature. The mortality rate is less in the October- November session. In summer the rate increases up to 40%. However, on application of the compound $[Cu(val)_2(H_2O)]$ **(10)**, the mortality rate is found to reduce by 10% only while in October – November session it reduces to 2% only. After the complete cycle, the eggs laid by the silk moth, both the control and treated were allowed to form larva. The quantity of the eggs laid is almost same. The process was repeated three times and the average values of larval weights are reported in **Table. 6.3**. The findings on the wet body weight(BW) during the developmental stages in the 5th instars of *(Samia cynthia ricini)* are presented in **Table 6.4.** The results are comparable to those obtained earlier [**47-48**].

Table 6.3: Day wise weight of larva *(Samia cynthia ricini)*

*** Control**		***Treated**	
Days	Average larval weight (g)	Days	Average larval weight (g)
1	2.5	1	2.5
2	3.5	2	3.6
3	4.3	3	4.5
4	5.2	4	7.8
5	6.0	5	8.7
6 **6.30AM**	3.4	6.5 **6.30PM**	6.9

*commuted average data for experiments conducted in: June-July' 2009, December 2009-January 2010, and March 2010 - April 2010

Table 6.4 : Comparative growth of the larva in its 5th instars for control and treated

Control		**Treated**	
Days	Average larval weight (g)	Days	Average larval weight (g)
1	2.5	1	2.5
2	3.5	2	3.6
3	4.3	3	4.5
4	5.2	4	7.8
5	6.0	5	8.7
6 6.30AM	3.4	6.5 6.30PM	6.9

The body weight(BW) of the (*Samia cynthia* ricini) - eri silkworm larvae increases several fold from first instar till maturation. In each larval instar, the BW increased significantly towards late larval instar with a decline in moulting. It increased exponentially from 1st instar to 5th instar and attained the peak in the late period of the latter stage. Expectedly, the weight of the female larvae at the last larval instar was significantly higher than that of the male counterparts (**Table 6.4**). However, the weight of both the sexes exponentially decreased during spinning and pupal stages. The increase in length of the body of the larva during the developmental stages in the 5th instars of the silkworm *(Samia cynthia ricini)* are presented in **Table.6.5.**

Table. 6.5. Day wise length of eri larva *(Samia cynthia ricini)*

***Control**		***Treated**	
Days	Average length of the larva (cm)	Days	Average length of the larva (cm)
1st	5.8	1st	5.8
2nd	6.0	2nd	6.0
3rd	6.5	3rd	6.2
4th	6.6	4th	7.1
5th	6.8	5th	7.1
6th 6.30am	6.5	6th 6.30pm	7.0

* commuted average data for experiments conducted in : June- July' 2009, Dec'09- January2010, and March 2010- April 2010

It is found that the application of mixedligand complexes of zinc-fluoro-aminoacid enhances the growth of the worm as well as the shell weight compared to the control species (insect). The results shows that, a 1% aqueous solution of $NH_4[ZnF(ser)_2(H_2O)].5H_2O$ **(14)** ; 5 µL, just after 5-10 hour of the fifth instar when applied to the insect, increases both the silkworm weight and silk about 150% (**Fig.6.1, Table 6.6**). The average shell weight of the control species in the month of December is found to be 0.40 g and the application of the newly synthesized compound $NH_4[ZnF(ser)_2(H_2O)].5H_2O$ **(14),** 5µl of 1% aqueous solution to the insect in its fifth instar increases this up to 0.86g and the average shell weight to 0.76 g. Moreover the average shell weight of eri silkworm in Sericulture farm at Diphu in the month of December and February was also found to be 0.40g, similar to the weight of the controlled species. Thus application of only 5µL of 1% aqueous solution of the compound, $NH_4[ZnF(ser)_2(H_2O)].5H_2O$ **(14)** as a food on the castor leave can enhance of production of silk by about 200% in *Samia cynthia ricini*. The extent

of enhancement observed is quite unprecedented considering the related literature on chemical induced improvement of silk production by silkworm. It is important to note that the insect themselves takes initiative to have the compound. When the compound was applied on the castor leaves, the insect immediately finish the part of the leave treated with the compound. Studies related to such external supplementation triggered improvement of silk production are mostly confined to *B. mori* species and those dealing with *S. ricini* are very few.

Similar studies involving application of $[Cu(val)_2(H_2O)]$ **(10)** has been carried out, however, no significant increase in silk production by the eri silkeworm was observed. Rather, it is found that the mortality rate of the insect is significantly reduced and ten out of ten larvae were found alive till the stage of moth.

Larva produced from egg of eri silkworm*(Samia cynthia ricini)* changes their instars five time in their life cycle. During this period they feed on green leaves except the time of their change of instars. Highest growth was noticed in their 5^{th} instars when they produces silk in their silk glands. A full grown larva roam around to find a suitable dry place to form cocoon . They release silk fiber and spin to form cocoon surrounding their body. Inside the cocoon they stay for one month and then come out from the cocoon as a moth. When they pair, they lay egg. The photographs in **Fig.6.1(a-f)** represents the life cycle of the insect.

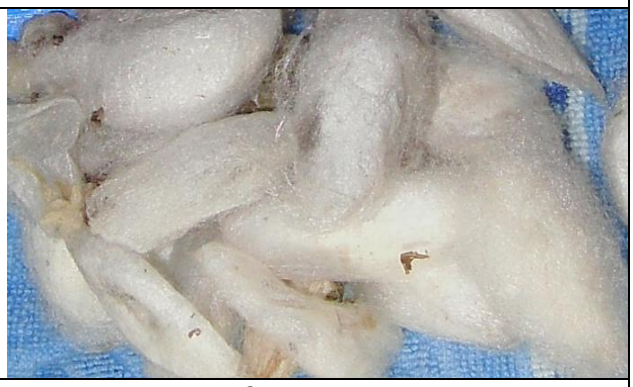
a. Eri larva coming out from eggs (DFL)

b. Growing larva feeding on castor leaves

c. Changes their instars

d. Larva in roaming

e. Spinning



f. Cocoons

g. Moth from cocoon

h. Eri moth

Fig.6.1. The life cycle of eri silkworm (**a-h**)

It is observed that the duration of 5th instars of the eri silkworm increases by 12 hours when treated with 5µL of 1% aqueous solution of the compound $NH_4[ZnF(ser)_2(H_2O)].5H_2O$ **(14)** as a food on the castor leave (**Fig. 6.2**).This is believed to be the crucial step towards the improved production of silk.

Test compound applied(5µL, 1% aqueous solution)	Silkworm spinning (control),(wt.=3.4g) .	Silkworm feeding on castor leave (treated) (wt.=6.4gm)

Fig. 6.2: Photographs showing some stages during growth

The research result shows that, using 1% aqueous solution of $NH_4[ZnF(ser)_2(H_2O)].5H_2O$ **(14)**; 5 µL, just after 5-10 hour of the fifth instars to the insect *(Samia cynthia ricini)* rapidly increases the silk gland weight giving higher production of silk. Dissection of two larva (control and treated) are shown in **Fig.6.3.**

Control	**Treated**
	
Weight of single silk gland = 1.80gm (completely mature) 9/1/10 - 6.30am	Weight of single silk gland = 2.36gm (not completely mature) 9/1/10 - 6.30am

Fig.6.3 The silk gland weight of eri silkworm (control & treated)

The weight of cocoon, pupa, silk (also see **Fig.6.4)** and moth are reported in **Table 6.6** for the **(14)** treated cases reflecting more than 1.5 fold increase in all the parameter studied.

Table 6.6: Weight of cocoon, pupa, silk and moth for control and treated

Species	¥Weight (g)			
	Cocoon	pupa	Silk	*Silk moth
Control	2.7±0.2	2.3±0.2	0.28 ± 0.2	0.52 ± 0.04
Treated	4.3±0.4	3.5±0.3	0.76 ± 0.4	0.75 ± 0.04

*After the exit of the silk moth.
¥ Values are mean of 3 replicates of ten cocoons.

For determination of shell ratio percentage (SR %) (percentage of shell weight : cocoon weight), five healthy cocoons were selected from control and treated **(14)** groups and the cocoon weight, shell weight were recorded (**Table 6.7**).

Table 6.7 : Cocoon weight, shell weight and shell ratio (SR%*)

Control					**Treated**				
Cocoon wt.	Average	Shell wt.	Average	SR %	Cocoon wt.	Average	Shell wt.	Average	SR %
2.47		0.44			4.12		0.76		
2.61		0.40			4.69		0.82		
2.79	2.652	0.38	0.392	14.78	3.96	4.087	0.72	0.75	18.35
2.54		0.37			3.80		0.70		
2.85		0.37			3.82		0.75		

* SR **%** (Cocoon weight: shell weight ratio) # all weights are expressed in gram

The shell% ratio of 18.35 obtained in this experiment compares well with many other dietary enrichment studies carried out on different silkworm species[**51-55**]. The **Table 6.5**, **Table 6.6 and Table 6.7** furnishes the comparative account of some economic parameter of control and treated cases reflecting almost a two-fold increase. The weight of treated larvae increases sharply from the control registering 100% hike from the 3rd day of the 5th instars (**Fig.6.5)**. Tanaka observed an 80% increase in the larval body weight during the 5th instar in *B. Mori* [**56**]. A similar trend, little lower though, was also observed by Reddy and Benchamin for different species [**57**]. Lack of studies on *Samia ricini*, as already mentioned, precluded any comparisons with this species.

Control	Treated
	
	
	

Fig.6.4. The weight of silk (control and treated, three replicates)

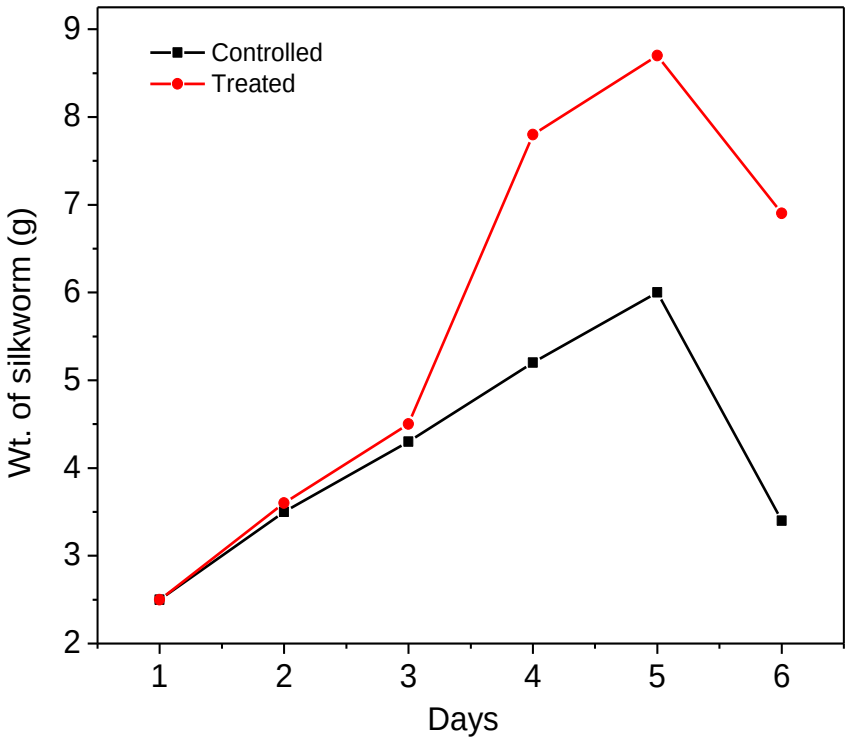


Fig.6.5. Plot of variation of silkworm weights versus days

Cumulatively, the findings of the present study suggest that zinc-aminoacid complex **(14)** induced active turnover of all profiles of protein metabolic events in the posterior silk gland, created conditions that are highly congenial for growth and silk production in eri silkworm. Simultaneous application of zinc and amino acid in the form of a single compound is thought to be the reason for the observed enahancement in silk productivity. Though role of fluoride is not yet clearly understood.

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