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Effect of Nanoparticles on Cocoon Parameters of Eri Silkworm, *Samia cynthia ricini* (Boisduval)

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ABSTRACT

Sericulture is the practice of raising silkworms to produce raw silk. Synthetic pesticides have significantly increased in use for crop protection during the past 50 years. The growing concern for the environment and human health has heightened the need for novel low-risk control techniques and new chemical classes of pesticides. Recently adopted nanotechnology in the pest management industry has the potential to alter contemporary agriculture. Understanding their toxicity on non-target species is necessary due to the rapid growth of nanomaterials in several scientific domains. In the present study, Chitosan, Silver (Ag) and Zinc oxide (ZnO) nanoparticles (NPs) at seven different concentrations (25, 50, 100, 200, 300, 400, 500 ppm) were used on Eri silkworm (*Samia cynthia ricini,* Boisduval). The cocoon weight of the larvae treated with Zinc oxide nanoparticle showed little to no difference with control however the cocoons treated with Chitosan and Silver nanoparticle exhibited decreased cocoon weight with increasing concentration of nanoparticle treatment. The fibroin protein percentage decreased while the sericin protein percentage increased with increasing concentration of all the three nanoparticle treatments. The defects in the silk threads were observed highest at 500 ppm of Chitosan nanoparticle in all the defect types (Loop, Bad casting, Split end, Slug and Waste) which was followed by Silver and Zinc oxide nanoparticle in Split end, Slug and Waste while it was followed by Zinc oxide in Loop and Bad casting. The present study indicates toxic effects of nanoparticles on the cocoon parameters of beneficial insect like Eri silkworm.

1. Introduction

Silkworm is a member of the order Lepidoptera, family Saturniidae with substantial economic value. It goes through four developmental stages: eggs, larvae, pupae and adults. (Truman and Riddiford, 1999). The silkworms secrete silk, "the textiles Queen". One of the most expensive natural fibres is silk, with the unit cost of raw silk around 20% higher than that of raw cotton. India is unique in producing all the four commercial varieties of silk namely Mulberry (*Bombyx mori*), Tasar (*Antheraea proylei*), Muga (*Antheraea assamensis*) and Eri (*Samia cynthia ricini*). Sericulture is the ideal cottage industry due to its labor-intensive nature, industrial overhang and rural roots. It stands out for requiring little startup capital and yielding quick and significant returns, making it the ideal business that befits India's socioeconomic system. It has gained recognition as an important economic sector in India because of its ability to strengthen the rural economy, create job opportunities and increase export earnings.

The multivoltine Eri silkworm, *Samia cynthia ricini* (Boisduval), has five to six generations per year. The only fully domesticated non-mulberry silkworm is the Eri silkworm, sometimes referred to as errandi or endi silk. They consume a variety of host plants, primarily those in the families *viz.* Euphorbiaceae, Araliaceae, Apocynaceae, and Simarubiaceae and are polyphagous (Chowdhury, 1982). Castor (*Ricinus communis* L.) is the most preferred food plant for rearing of Eri silkworm (Ghose, 1949; Fukuda *et al.*, 1961). Kesseru (*Heteropanax fragans*) is utilized next in India is the world's biggest producer of Eri silk, accounting for 96%

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of global production. The Eri silk industry has a special place for India's northeast. Eri silk, also known as "poor man's silk," is more widely used throughout Assam. It has the feel of wool, is as soft as silk and resembles cotton. These host plants of Eri silkworm can be attacked by various pests as well as diseases and thus use of synthetic, chemical pesticides can be used as a go-to remedy for such attacks.

Synthetic pesticides are used all over the world to safeguard crops and prevent diseases in humans, animals, and plants. Their use has grown steadily during the past 50 years. However, the increased focus on environmental and human issues has increased demand for new, low-risk control strategies and pesticide chemical classes (Stadler *et al.* 2012). The ecosystem, farmers, consumers and non-target organisms all suffered from the increased usage of pesticides. India's post-Green Revolution regulations require that higher concentrations of fertilisers and pesticides be used less frequently in order to save future agriculture and the environment. The challenging task of establishing a balance between food production and environmental conservation can only be accomplished by utilising cutting-edge strategies like nanotechnology.

The term “nano” is derived from the Greek word “nanos” meaning dwarf (Sangamithra and Thirupathi, 2009). The word "nano" is more specifically defined as a measure of 10^{-9} m, or one billionth of a metre. Nanotechnology is the synthesis, characterization, exploration, and use of materials that are nanosized (1-100 nm) for the development of science. Nanoparticles have a larger surface area than macro-sized materials since they are smaller than those materials, which results in the introduction of unique features. Nanomaterials display distinctive characteristics that are absent from their bulk form due to their small size and high surface area to volume ratio (Powell and Kanarek, 2006). Through the controlled and targeted distribution of agrochemicals and the provision of early detection diagnostic instruments, the application of nanotechnology in plant protection has enormous promise for managing diseases and pests.

However, due to the rapid growth and spread of nanomaterials in the modern world, they may come into contact with living beings in ways that aren't fully understood. According to current research, nanomaterials exhibit different hazardous characteristics from their bulk versions because of their small size and high reactivity (Brayner *et al.,* 2010). An increased need to examine how nanomaterials affect the environment in general and insect and plant life in particular has resulted from the usage of nanomaterials in a variety of scientific domains (Yasur and Pathipati, 2015). In light of the rapid advancement of nanomaterials in many scientific fields, it is essential to comprehend the adverse consequences of application of nanoparticles on the growth and physiology of non-target species and the environment. Though the use of nanoscaled materials is quickly expanding, their precise potential consequences on the environment and toxicology are still unknown (Panacek *et al.,* 2011). Keeping the non-target organisms in mind the silkworms being domesticated can be major consumers of these chemicals directly through their host plant leaves which is their only source of diet. Any toxic effect of these chemicals on the commercial significance can impact negatively for the rearers which will decrease their market value. Thus, it is important for us to understand the toxic effect of such nanoparticles on silkworms, specifically Eri because of its significance in the North-east region of the country.

2. Materials and Methods

Rearing of the silkworm larvae

Fifteen disease free layings (DFLs, 1 DFL = 300-350 eggs) of Eri silkworm eggs were collected from District Sericulture Office at Nongpoh, Ri-Bhoi, Meghalaya under Directorate of Sericulture and Weaving, Government of Meghalaya for rearing in winter (November-December, 2022) for the present study. The DFLs of eggs collected were subjected to surface sterilization with 2% formalin solution and washed with clean water and dried under shade. The eggs were cleaned thoroughly and kept on clean paper for incubation at room temperature till hatching. The first instar greenish yellow newly emerged larvae were fed with very tender leaves of Kesseru. The subsequent instars of the silkworm larvae were fed with tender to mature Kesseru leaves till they complete their 5^{th} instar. The 5^{th} instar larvae after 5-6 days stopped feeding and became inactive and entered ripening stage. The larvae were then collected from the sides of tray and kept in mountages for them to pupate and form cocoon. With 15 DFLs of eggs there were a total of 4,500 (approx.) eggs used in the study. Total numbers of treatments were 8 with 3 replications each in all the 3 nanoparticle treatments. Thus, the total number of larvae used in each rearing tray was 60 (approx.).

Preparation of different concentrations of nanoparticles

The stock nanoparticle of all the three nanoparticles used in the study (Silver, Chitosan and Zinc oxide) were collected from School of Crop Protection, College of Post Graduate Studies in Agricultural Sciences, Central Agricultural University (Imphal), Umiam, Meghalaya. The different concentrations of nanoparticle treatments were made by dilution with 100 ml of sterile distilled water into seven different concentrations *viz.* 25, 50, 100, 200, 300, 400, 500 ppm each.

Application of the nanoparticle treatment

The nanoparticles (NPs) were applied by leaf dip method on the Kesseru leaves which were kept on the rearing trays for the larva to feed on. The tender leaves of Kesseru were used for feeding of the 1st and 2nd instars and the treatments were applied on them. These leaves were then left to air dry and then placed gently over the larva on the rearing trays for them to feed on. The mature leaves of Kesseru were used to feed 3rd, 4th and 5th instar larva. The treatments were applied on the leaves once every instar. The 1st application was done 2-3 days after all the larva hatched from the eggs and they were separated into different rearing trays for the treatments.

Parameters considered

a. Cocoon weight:

The weight of each cocoon was recorded (in grams) with the help of an electronic open pan balance. The cocoon weight of each of the treatment and also from each of the replication was recorded and noted down separately for further analysis of the data.

b. Silk quality assessment:

The quality of the silk in the cocoons developed from different concentrations of the nanoparticles was assessed. The cocoons were collected and their individual silk thread in 1 cm^2 area was observed under Stereo zoom microscope and raw silk testing was carried out which included cleanness test to check for major and minor defects in the silk like wastes, large slugs, bad casts, long knots, heavy cork screws, split-ends and long loops in the thread as suggested in Silk reeling and testing manual by Yong-woo Lee (1999).

c. Protein percentage in the cocoon:

Fibroin and Sericin was estimated by following the procedures suggested by Orlandi (1954):

Known quantity of cocoon shells were treated with 2 per cent KOH solution and boiled for 3 to 4 minutes at 70 to 80° C. The pluffy cocoon shells were then washed thoroughly in tap water to remove the traces of alkali and further the pluffy cocoons were dipped in diluted acetic acid (1 g/l) for few minutes to neutralize the alkali present in the cocoon shells. After another thorough wash in water the pluffy cocoons were dried at 90 to 100° C in hot air oven for few minutes. The weight of fibroin thus obtained after dissolution of sericin was recorded. The fibroin and sericin contents in cocoon shells were calculated using formulae:

$$\text{Fibroin (\%)} = \left[\frac{\text{Weight of pluffy shells (g)}}{\text{Weight of initial cocoon shell (g)}}\right] \times 100$$

$$\text{Sericin (\%)} = 100 - \text{Fibroin (\%)}$$

Statistical data analysis

The current study included 8 treatments with 3 replications each for all the 3 nanoparticle treatments. The experimental design used was Completely Randomized Design (CRD). Data was subjected to analysis of variance using standard statistical procedure (Gomez and Gomez, 1984) for CRD. Correlation analysis was done by finding out the correlation coefficient, which was calculated.

3. Results

Effect of different concentrations of nanoparticles on weight of cocoon

The cocoon weight of larvae treated with Chitosan, Silver and Zinc oxide nanoparticles decreased with increased concentration of the treatment with lowest cocoon weight observed at 500 ppm except for Zinc oxide treated larvae. The pupae from larvae treated with 500 ppm of nanoparticles had significant difference of cocoon weight of 1.943, 2.027 and 2.833 g/cocoon of Chitosan, Silver and Zinc oxide with control (2.833 g/cocoon) (Table 1).

Table 1. Effect of different concentrations of Chitosan, Silver and Zinc oxide nanoparticles on cocoon weight

Treatment	Cocoon weight (in g/cocoon)		
	Chitosan NPs	Silver NPs	Zinc Oxide NPs
Control	$2.83^{a} \pm 0.10$	$2.83^{a} \pm 0.10$	$2.83^{a} \pm 0.10$
25 ppm	$2.85^{ab} \pm 0.07$	$2.96^{ab} \pm 0.06$	$2.98^{b} \pm 0.14$
50 ppm	$2.79^{ab} \pm 0.02$	$2.86^{bc} \pm 0.05$	$2.86^{b} \pm 0.01$
100 ppm	$2.60^{b} \pm 0.06$	$2.74^{cd} \pm 0.05$	$2.86^{b} \pm 0.04$
200 ppm	$2.73^{c} \pm 0.07$	$2.66^{de} \pm 0.03$	$2.81^{bc} \pm 0.02$
300 ppm	$2.54^{c} \pm 0.04$	$2.52^{e} \pm 0.01$	$2.82^{bcd} \pm 0.04$
400 ppm	$2.40^{d} \pm 0.02$	$2.50^{e} \pm 0.07$	$2.72^{cd} \pm 0.02$
500 ppm	$1.94^{e} \pm 0.06$	$2.027^{f} \pm 0.07$	$2.83^{d} \pm 0.10$
SE(m) ±	0.034	0.034	0.036
CD (p=0.05)	0.102	0.103	0.109

Effect of different concentrations of nanoparticles on defects of silk thread

According to Yong-woo Lee (1999) defects observed in silk threads were categorized as per the following:

- Split end – When the silk end has split into two ends.
- Waste – Congregation of the silk threads which are tightly bound.
- Slug – Congregation of the silk threads which are loosely bound.
- Bad casting – Lumps on the silk thread.
- Loop – Circular, loop like structures between the silk threads.

The defects found on the individual silk threads observed under Stereo zoom microscope increased with increased concentrations of nanoparticles with highest defects seen at 500 ppm of Chitosan, Silver and Zinc oxide nanoparticle in 1 cm^2 area of a cocoon. The number of split end defect was observed highest at 500 ppm of Chitosan (10.11) followed by Silver (9.00) and Zinc oxide (4.33) nanoparticle. The number of slug defect was observed highest at 500 ppm of Chitosan (9.00) followed by Silver (6.00) and Zinc oxide (3.67) nanoparticle. The number of bad casting defect was observed highest at 500 ppm of Chitosan (11.33) followed by Zinc oxide (8.00) and Silver (5.00) nanoparticle. The number of waste defect was observed highest at 500 ppm of Chitosan (6.67) followed by Silver (4.00) and Zinc oxide (3.67) nanoparticle. The number of loop defect was observed highest at 500 ppm of Chitosan (8.67) followed by Zinc oxide (6.00) and Silver (3.67) nanoparticle (Table 2).

Effect of different concentrations of nanoparticles on protein content in the cocoons

The cocoons contain two types of protein namely, Fibroin and Sericin. The Fibroin protein is found in the inner side of the cocoon which allows for tight packaging of the sheets which accounts for rigidity and high tensile strength in the silk. Sericin protein is found on the outer part of the cocoon which helps in binding the cocoon strands together to keep it intact. The Fibroin protein content in the cocoons decreased with increased concentrations and with 500 ppm, the corresponding values were 68.03%, 71.13% and 69.49% in respect of Chitosan, Silver and Zinc oxide respectively as compared to control (77.92 %). The Sericin protein content in the cocoons increased with increased concentrations and with 500 ppm, the corresponding values were 31.97%, 28.87% and 30.51% in respect of Chitosan, Silver and Zinc oxide as compared to control (22.08 %) (Table 3).

Table 2: Effect of different concentrations of Chitosan, Silver and Zinc oxide nanoparticles on silk thread defects

TREATMENT		SPLIT END			SLUG			BAD CASTING			WASTE			LOOP		
		Chi. NPs	Ag NPs	ZnO NPs	Chi. NPs	Ag NPs	ZnO NPs	Chi. NPs	Ag NPs	ZnO NPs	Chi. NPs	Ag NPs	ZnO NPs	Chi. NPs	Ag NPs	ZnO NPs
T_1	Control	1.33	1.54	1.21	1.40	1.22	1.10	1.89	1.73	1.66	1.03	0.72	0.33	1.92	0.67	0.88
T_2	25 ppm	2.89	3.67	2.33	4.00	3.67	1.67	3.67	2.00	2.67	1.00	2.67	2.00	3.67	2.33	2.67
T_3	50 ppm	4.11	3.00	4.00	2.67	5.00	4.00	4.67	5.00	3.33	2.33	4.67	2.67	3.00	4.67	3.00
T_4	100 ppm	6.00	5.67	3.33	5.67	7.00	3.33	5.00	5.67	4.33	2.00	5.00	2.33	5.00	3.67	3.00
T_5	200 ppm	6.89	4.33	4.00	2.67	7.00	4.00	6.33	5.00	4.00	3.00	5.33	3.00	6.33	5.67	1.67
T_6	300 ppm	6.33	6.33	5.00	6.33	6.33	3.67	7.33	6.33	5.67	4.67	7.00	4.00	6.00	5.33	2.00
T_7	400 ppm	8.11	7.33	5.67	8.67	6.33	5.00	8.67	7.33	7.00	7.00	6.00	4.00	7.00	5.67	4.00
T_8	500 ppm	**10.11**	**9.00**	**4.33**	**9.00**	**6.00**	**3.67**	**11.33**	**5.00**	**8.00**	**6.67**	**4.00**	**3.67**	**8.67**	**3.67**	**6.00**

SE(m)±	0.68	0.73	0.59	1.25	0.82	0.62	0.96	0.82	0.80	0.62	0.79	1.07	0.96	0.82	0.56
$CD_{(p=0.05)}$	2.04	2.18	1.77	3.74	2.44	1.87	2.89	2.45	2.39	1.87	2.37	3.20	2.89	2.47	1.69

Table 3: Effect of different concentrations of Chitosan, Silver and Zinc oxide nanoparticles on protein content of cocoons

Treatments		Protein content of cocoons (%)					
		Chitosan NPs		Silver NPs		Zinc Oxide NPs	
		Fibroin (%)	Sericin (%)	Fibroin (%)	Sericin (%)	Fibroin (%)	Sericin (%)
T_1	Control	77.92	22.08	77.92	22.08	77.92	22.08
T_2	25 ppm	78.55	21.45	79.28	20.72	78.30	21.70
T_3	50 ppm	77.90	22.10	78.68	21.32	77.59	22.41
T_4	100 ppm	76.34	23.66	78.49	21.51	78.49	21.51
T_5	200 ppm	77.81	22.19	77.67	22.33	76.97	23.03
T_6	300 ppm	76.91	23.09	78.54	21.46	76.94	23.06
T_7	400 ppm	72.52	27.48	73.26	26.74	72.35	27.65
T_8	500 ppm	**68.03**	**31.97**	**71.13**	**28.87**	**69.49**	**30.51**
SE(m) ±		0.799	0.799	1.523	1.523	1.410	1.410
$CD_{(p=0.05)}$		2.397	2.397	4.566	4.566	4.226	4.226

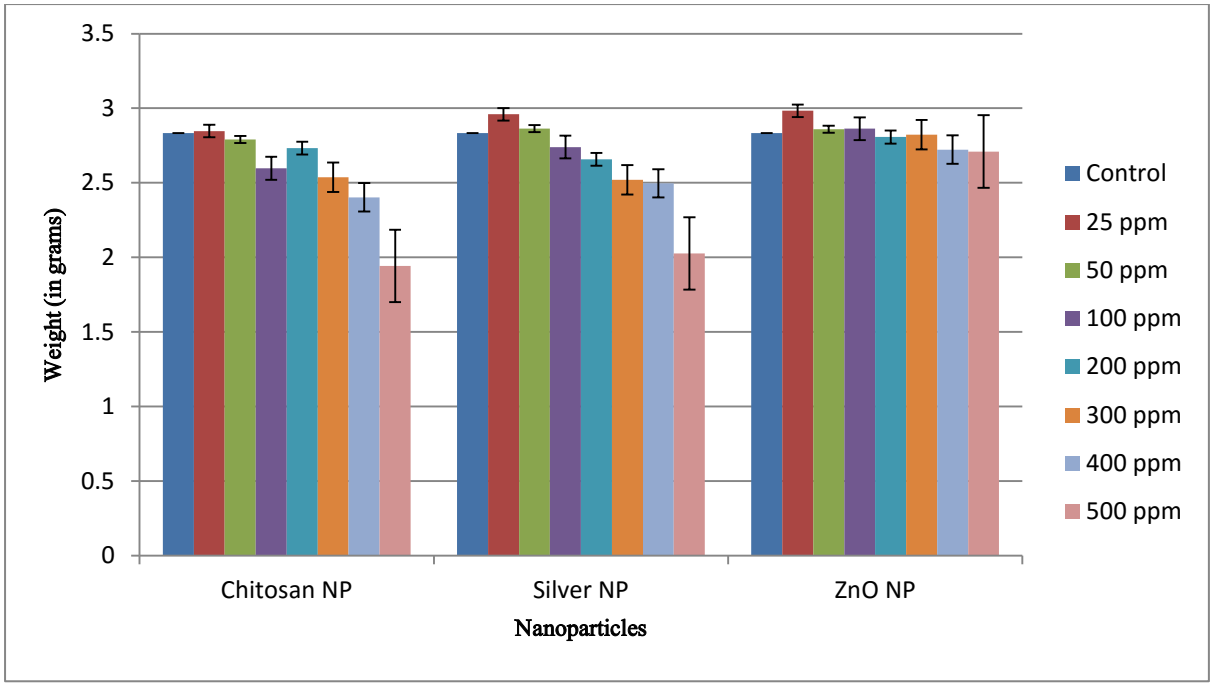

Fig. 1: Effect of different concentrations of Chitosan, Silver and Zinc oxide nanoparticles on cocoon weight

Plate 1: Cocoons showing difference in size; a: Control; b: Chitosan (500 ppm); c: ZnO (500 ppm) and d: Ag NPs (500 ppm)

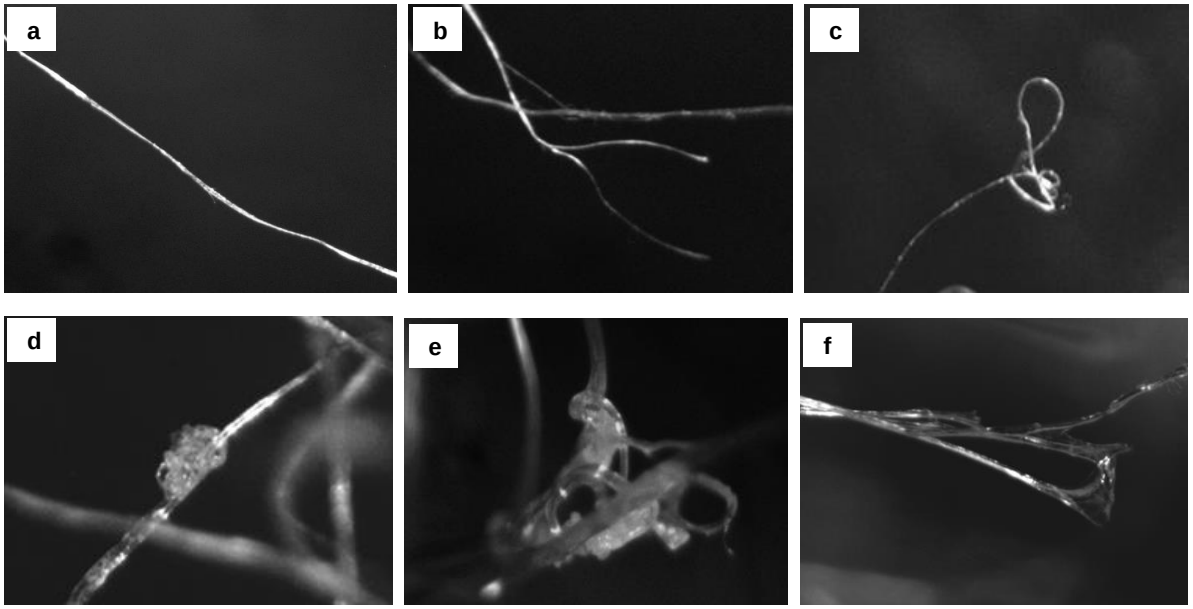

Plate 2: Defects in silk thread a: Control, b: Split end c: Slug, d: Bad casting, e: Waste, f: Loop in Chitosan, Ag and ZnO nanoparticles (500 ppm).

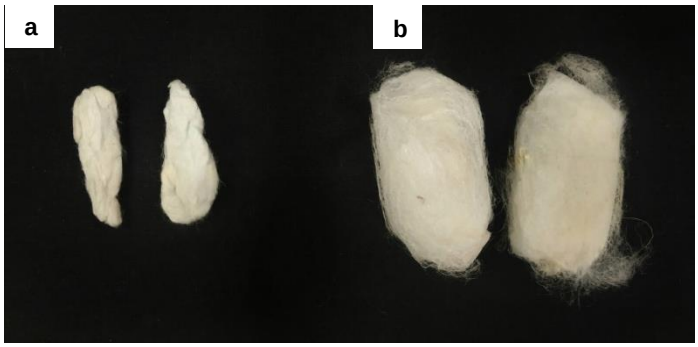

Plate 3. Cocoon protein content assessment; a: Oven dried pluffy cocoons and b: normal cocoons.

4. Discussion

The cocoon weight of the pupae whose larvae were treated with Zinc oxide nanoparticle exhibited little to no variation as compared to control while the cocoon weight due to treatment with Chitosan and Silver nanoparticle significantly deceased with increased concentration of the nanoparticles. The cocoon weight showed no variation from 25 to 200 ppm of all the three nanoparticles but at 300, 400 and 500 ppm of Chitosan (2.54, 2.40 and 1.94 g/cocoon) and Silver (2.52, 2.49 and 2.03 g/cocoon) nanoparticles it exhibited significant difference as compared to control (2.83 g/cocoon). The cocoon weight obtained due to treatment with Zinc oxide nanoparticle at 300, 400 and 500 ppm (2.82, 2.72 and 2.83 g/cocoon) showed no difference with the control (2.83 g/cocoon). Similar results were presented by Panacek *et al.* (2011) where *Drosophila melanogaster* when exposed to Silver nanoparticle exhibited weight loss of 24% as compared to control.

The silkworms contain two proteins in their cocoons (Fibroin and Sericin) which provide strength and adherence to the silk threads. The cocoons treated with different concentrations of Chitosan, Silver and Zinc oxide nanoparticles exhibited decrease in Fibroin % with increased concentrations of the nanoparticles with the lowest observed in pupae treated with 500 ppm of Chitosan (68.03 %) followed by Zinc oxide (69.49 %) and Silver (71.13 %) nanoparticles. While it was noted that there was an increase in Sericin % with increased concentrations of the nanoparticles with highest observed in pupae treated with 500 ppm of Chitosan (31.97 %) followed by Zinc oxide (30.51 %) and Silver (28.87 %) nanoparticles. No work has been found on similar grounds to support the findings, thus further studies would furnish us with greater and enhanced data on the effects of the nanoparticles on the cocoon protein content.
The silk threads of pupae, whose larvae were treated with different concentrations of Chitosan, Silver and Zinc oxide nanoparticle exhibited increase in defects in the silk threads with increased concentrations of the nanoparticles. The defects were observed highest at 500 ppm of Chitosan nanoparticle in all the defect types (Loop, Bad casting, Split end, Slug and Waste) which was followed by Silver and Zinc oxide nanoparticle in Split end, Slug and Waste while it was followed by Zinc oxide in Loop and Bad casting. The findings thus observed revealed that these nanoparticles might have some effect on the silk glands of the Eri silkworm thus producing more defects in the nanoparticle treated pupae as compared to the control pupae. Since with increase in concentration of nanoparticles the sericin protein % of the cocoons also increased with highest being at 500 ppm. So, a conclusion can be drawn that as Sericin protein is found on the outer part of the cocoon which helps in binding the cocoon strands together to keep it intact this can be a reason for increased defects with increased concentration of nanoparticles since there might be accumulation of sericin protein in the outer covering of the cocoon. No work has been done on similar grounds to support the findings, thus further studies would provide better and improved information about the effect of nanoparticles on the defects of silk thread in Eri silkworm.

5. Summary and Conclusion

- The cocoon weight decreased with increased concentration being lowest at 400 and 500 ppm of Chitosan (2.40 and 1.94 g/cocoon) and Silver (2.50 and 2.03 g/cocoon) nanoparticle as compared to the control (2.72 and 2.83 g) while pupae treated with Zinc oxide nanoparticle (2.83 g/cocoon) was at par with the control (2.83 g/cocoon).
- The pupae of the larvae treated with Chitosan, Silver and Zinc oxide nanoparticles exhibited defects in the silk thread which increased with increased concentration with highest defects observed at 500 ppm in all the three nanoparticles:
    - Split end defect was highest in Chitosan (10.11), followed by Silver (9.00) and Zinc oxide (4.33) nanoparticles as compared to control with 1.33, 1.54 and 1.21 in Chitosan, Silver and Zinc oxide nanoparticle, respectively.

- Slug defect was highest in Chitosan (9.00), followed by Silver (6.00) and Zinc oxide (3.67) nanoparticles as compared to control with 1.40, 1.22 and 1.10 in Chitosan, Silver and Zinc oxide nanoparticle, respectively.
- Bad casting defect was highest in Chitosan (11.33) followed by Zinc oxide (8.00) and Silver (5.00) nanoparticles as compared to control with 1.89, 1.73 and 1.66 in Chitosan, Silver and Zinc oxide nanoparticle, respectively.
- Waste defect was highest in Chitosan (6.67) followed by Silver (4.00) and Zinc oxide (3.67) nanoparticles as compared to control with 1.03, 0.72 and 0.33 in Chitosan, Silver and Zinc oxide nanoparticle, respectively.
- Loop defect was highest in Chitosan (8.67) followed by Zinc oxide (6.00) and Silver (3.67) nanoparticles as compared to control with 1.92, 0.67 and 0.88 in Chitosan, Silver and Zinc oxide nanoparticle, respectively.

- The Fibroin protein content in the cocoons decreased with increased concentrations with lowest observed in 500 ppm of Chitosan (68.03 %) followed by Silver (71.13 %) and Zinc oxide (69.49 %) as compared to the control (77.92 %).
- The Sericin protein content in the cocoons increased with increased concentrations with highest observed in 500 ppm of Chitosan (31.97 %) followed by Silver (28.87 %) and Zinc oxide (30.51 %) as compared to the control (22.08 %).

Based on the results of the present study Chitosan, Silver and Zinc oxide nanoparticles exhibited nano-toxic effects on beneficial insect, Eri silkworm at concentrations of 400 ppm and higher. The toxicity of nanoparticles resulted in morphological deformities in the cocoon parameters. Thus, 400 ppm and above concentration is regarded as harmful for rearing of Eri silkworm due to nanoparticle toxicity in their morphology which reduces their commercial value. Nanoparticle concentrations upto 300 ppm even though exhibits slight morphological alterations but are not consequential enough as compared to the control. So, Chitosan, Silver and Zinc oxide nanoparticles upto a concentration of 300 ppm were found to be safe for all the post-cocoon parameters in Eri silkworm.

6. Acknowledgment

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7. Conflict of Interest

The authors declare that they have no conflict of interest within themselves and others including the agency where the research was carried out.

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