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Effect of Leaf and Seed Extracts of *Trichosanthes cucumerina* L. on Antibacterial Activity and Commercial Parameters of Silkworm *Bombyx mori* L.

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Abstract: The effect of leaf and seed extracts of *Trichosanthes cucumerina* L. was studied on the bacteria which infect the larva of silk worm (*Bombyx mori*). The same extracts were also used to know its influence on VIth instar larvae of silkworm for improving the silk gland and cocoon characteristics by feeding the treated mulberry leaves. The extracts of leaf and seed with methanol had shown maximum effect on almost all the bacteria tested. The various concentrations (T_1=1:1, T_2=1:2,T_3=1:3) of methanolic extracts of leaf and seed of *T. cucumerina* were administered on VIth instar silkworm and also they were fed with treated leaves of mulberry. The methanolic leaf and seed extracts have enlarged the development of silk gland linear length of silk gland of *B. mori* at the days. The plant extract of T_1 (both seed and leaf) had shown maximum ZI against all the gram positive and gram negative bacteria and influenced the larval and cocoon characteristics of *B. mori*, whereas T_3 concentration had shown lowest ZI except against *S. albus*, while T_2 concentration recorded to be moderate on antibacterial activity as well as on cocoon and silk charecteristics. The mean cocoon weight (1.94±0.11, 2.1±0.12), pupal weight (1.54±0.08, 1.6±0.06), shell weight (0.39±0.01,0.45±0.01), silk percentage (20.31%, 23 %) and filament length (97±0.17, 100±0.12) were increased at the concentration of T_1 dietary supplementation over the control of both the plant extracts of leaf and seed respectively. In the present investigations moderate concentration of *T. cucumerina* extracts have shown promoting effect on commercial parameters of silkworm.

Key words: Mulberry · Plant extracts · Zone of Inhibition(ZI) · Silk gland and cocoon weight

INTRODUCTION

Plants act as the richest source of organic chemicals and phytochemicals that influence life and behaviour of several insects [1]. India is considered to be a rich emporium of medicinal plants in the world. Nature has provided impressive number of drugs which have been isolated from the medicinal plants.

Silkworm *Bombyx mori* L feed specifically on mulberry leaves as well as the osage, orange and lettuce. *Bombyx mori* is of great economic importance as a foreign exchange earner for many silk producing countries of the World [2]. As the foundation of sericulture, the silkworm plays a major role for economic survival of farmers and workers in the textile industry. It is human endeavor through art and culture in globalization for nearly 2000 years during the Silk Road Era [3]. Today the silkworm plays an important role in basic research, sericulture and biotechnology [4]. As we have studied the phytochemicals present in *T. cucumerina,* we have also experimented the effect of leaf and seed methanolic extracts on bacteria and economic parameters of silkworm.

The larvae (caterpillars) of *B. mori* are about 4–5cm long. They are variously– colored. The adults have 4 cm wingspan [5]. *Bombyx mori* produces a fluid in their silk gland, which is a modified labial gland that is forced through spinnerets in their mouth. This fluid hardens in the air to produce the silk thread that they wrap around themselves to form as a cocoon. It has been reported that the silk fiber is almost a pure protein fiber composed of sericin and fibroin [6]. Besides sericin, raw silk also contains wax matter, carbohydrates, fat, inorganic matter

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and pigment [7]. Silk fibroin secreted in the lumen of posterior silk gland (PSG) of *B. mori* consists of three protein components: High (H)–chain 350 k Da, low (L)–chain 26 kDa [9] and glycoprotein P25 30 kDa [8–11]. These three types of fibroin are common among different silk producing insects.

Isolation of secondary metabolites from the seed of *Trichosanthes cucumerina* showed that it contains anti bacterial, antiplasmodic, antifungal, anti insecticidal properties [12]. The root and fruit juice of *T. cucumerina* used as anti– inflammatory, anti–cancer agents [13]. The leaf extracts of *T. cucumerina* showed the antibacterial activity [14].

As there is no report of methanolic extracts of leaf and seed on bacteria commonly infected to silk worm and also on commercial characters of silk worm, in this communication the present study has been under taken for first time.

MATERIAL AND METHODS

Plant Material: The leaves and seeds of *T. cucumerina* were collected from the Research field, Department of Biotechnology, Kakatiya University. These are shade dried, later finely powdered, using an electric blender and stored in air tight containers for further use by following the earlier procedure [15].

Preparation of the Plant Extracts: The shade dried leaves and seeds were powdered individually and 25 g of each were used separately for the extraction with 150 ml methanol (80%) for 24 h in Soxhlet equipment and filtered through 0.45 μm membrane filter. These filtrates were evaporated under reduced pressure and dried in a rotator evaporator at 55°C. Dried extracts were stored in screw cap bottles at –20°C and used as stock. Further, the same was diluted using distilled water to arrive at different concentrations (T_1=1:1, T_2=1:2, T_3=1:3 (Plant extract (leaf/seed): water). Fresh extracts were prepared on every third day.

Anti Bacterial Activity: The used bacteria are *Bacillus cereus*– KUCC 23, *Bacillus subtilis*–KUCC 17, *Staphylococcus aureus*–MTCC 96 and *St. albus*–MTCC 96.

Preparation of Bacterial Nutrient Medium: A standardized 1 to 2×10^4 cfu/ml 0.5 MC Farland standards was introduced onto the surface of sterile agar plate and evenly distributed the inoculum by using a sterile glass spreader. Simultaneously 8 mm wells were prepared in the petriplate using a sterile cork borer. Fifty microliter of extract at a concentration of 200 mg/ml was introduced into each well. The agar plates were incubated aerobically at 37°C.

Preparation of Test Solution for Antibacterial Activity: A concentration of 250 mg/ml of each solvent extract of leaf and seed was prepared in DMSO (which did not influence the microbial growth). After 24h, the inhibition zones were forming, measured with a ruler and compared with the control well containing only DMSO and 10 mg/ml of Gentamycin as standard. The antibacterial activity was studied by measuring the zone of inhibition (ZI) around the disc.

Procedure for Treatment of Silk Worms: Ten dfls (disease free layings) of Bivoltine dihybrid were selected for the study. These were purchased from National Silkworm Seed Organization (NSSO), Bangalore, Karnataka, India and reared on V1 mulberry variety in rearing house at Sericulture unit, Department of Zoology, Kakatiya University, Warangal (TS). The silk worms were reared up to the end of fifth instar as per the standard rearing method suggested by Datta *et al.* [16] using package of practices by Krishnaswami *et al*. [2]. The larvae after second moult were selected for treatment and were divided into four experimental groups including control viz., T_1=1:1, T_2=1:2, T_3=1: (Plant extract (leaf/seed): water). Each group consisted of three replicates as with 300 larvae each. The stock solution (100 ml) was diluted with known quantity of distilled water and three different concentrations of plant extracts were prepared to serve as treatments (T_1, T_2, T_3). These solutions were sprayed on the required quantity of mulberry leaves with an atomizer. The sprayed leaves were shade dried to remove excess moisture and fed to silk worms. Mulberry leaves sprayed with distilled water served as control. Fortified leaves with T_1, T_2& T_3 concentrations, were given daily to the silk worms after second moult till the day of spinning.

Isolation of Silk Glands from Silk Worms: The silkworms hatch to form pin–prick size grey–black eggs and grow to a 3–inch long caterpillar within one month. When they are just about ready to spin, havin emptied out any undigested food. These worms are dropped into vinegar and salt solution. These caterpillars were then placed in the lethal bath for approximately 12 h.

To harvest the gut, with sharp knife simply cut the caterpillars open and isolate the silk glands which are very easy to recognize. Take the glands with both hands. Simply pull it, with an even and consistent force and pull out to a length of 10–18 inches. Wash them in warm soapy water and scrape this carne off easily with fingernails. We feel silk gland like soft, slick and slightly rubbery. Later transfer onto the slide and measure the length of the silk gland by scale.

Data Analysis: The data of all the parameters were statistically analysed by following the method [17]. The results were recorded after repeating the experiments three times The experimental results were expressed as mean ± standard error (SE) of 3n measurements. The statistical analysis of the data were carried out using student's t-test and the results were considered significant when $p<0.05$.

RESULTS

The effect of methanolic seed and leaf extracts of *T. cucumerina* were studied on antibacterial activity, development of silk gland and also on commercial characters of silkworm.

Effect of Leaf and Seed Extracts on Antibacterial Activity: The results on zone of inhibition (ZI) of different concentrations of leaf and seed extracts of *T. cucumerina* are presented in Table–1 and shown in fig. 1&2

At T_1 concentration, the methanolic extracts of leaf and seed of *T. cucumerina* showed maximum ZI against *Bacillus subtilis* in comparison to standard. While more inhibition was observed at T_3 concentration against *Staphylococcus albus* in both leaf and seed extracts. Regarding the formation of ZI may be dependent upon the phytochemicals present in the extracts. This is possibly due to the differences in chemical composition and structure of cell wall of the microorganisms. Among all the concentrations tested leaf extracts showed maximum ZI at T_1 concentration against *Bacillus subtilis* compared to all other leaf and also seed extracts. The leaf extracts have shown the maximum ZI (10.6mm) followed by the seed extracts (9.8mm) at T_1 concentration against the same microorganism. Lowest zone of inhibition was recorded at T_3 concentration of both leaf and seed extracts except against *S. albus*. According to the results, the extracts of *T. cucumerina* exhibited antibacterial activity against both gram (+) ve bacterial strains *S. albus* and *S. aureus* and gram (–) ve bacterial strains *B. subtilis and B. cereus* mediating the presence of a broad spectrum of antibacterial compounds in the plant (Plate–I, II, fig 1& 2).

Table 1: Effect of leaf and seed extracts of *T. cucumerina* on microorganisms infected to silk worm

	Leaf Extracts				Seed Extracts			
Concentration of seed extract	*Bacillus subtilis* ZI in mm±SE)[a]	*Bacillus cereus* (ZI in mm±SE)[a]	*Staphylococ cus albus* (ZI in mm±SE)[a]	*Staphylococ cus aureus* (ZI in mm±SE)[a]	*Bacillus subtilis* (ZI in mm±SE)[a]	*Bacillus cereus* (ZI in mm±SE)[a]	*Staphylococc us albus* (ZI in mm±SE)[a]	*Staphylococcus aureus* (ZI in mm±SE)[a]
T_1	10.6±0.8	5.0±1.2	5.6±0.5	7.7±0.1	9.8±0.6	4.6±1.5	5.1±0.8	7.6±0.1
T_2	8.1±0.4	5.1±0.4	5.3±0.3	6.4±0.2	8.0±0.5	4.8±0.6	5.0±0.6	6.2±0.2
T_3	7.0±0.7	3.9±0.2	5.8±0.4	7.1±0.2	6.5±0.7	3.6±0.4	5.6±0.5	7.1±0.2
Gentamycin	5.3±0.7	3.0±0.4	3.8±0.1	5.2±0.5	5.3±0.7	3.0±0.5	3.0±0.2	5.2±0.5

T_1=1:1; T_2=1:2; T_3=1:3; Data presented as mean of 3 readings; ZI = Zone of Inhibition; ±SE[a]=Mean±Standard error

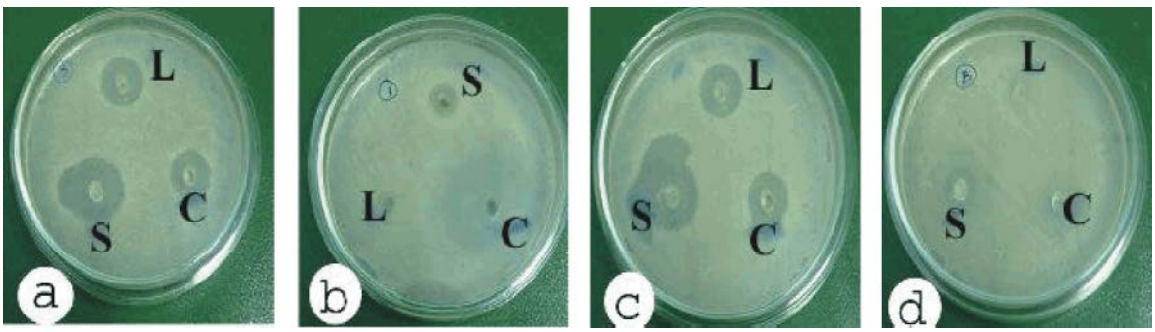

Fig. 1: a–d. Antibacterial activity of leaf extracts of *T. cucumerina* showing zone of Inhibition (in mm): a) *Bacillus cereus;* b) *Staphylococcus aureus;* c) *Bacillus subtilis;* d) *Staphylococcus albus* (C=Control; L=Leaf extract; S=Standard

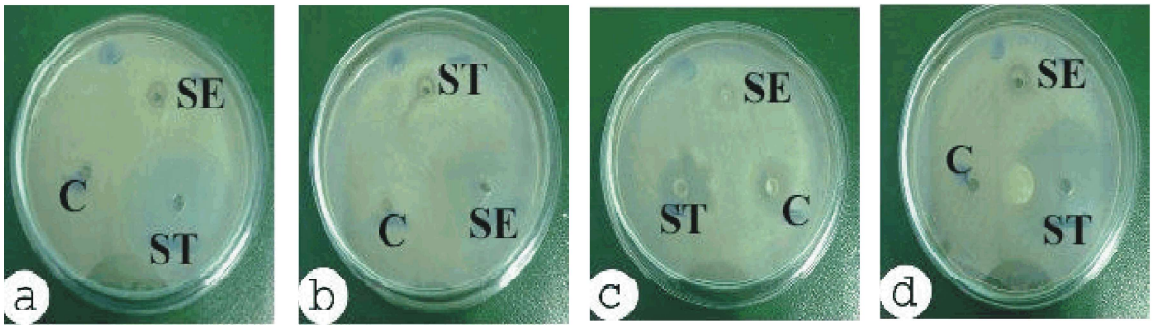

Fig. 2: a–d. Antibacterial activity of seed extracts of *T. cucumerina* showing zone of Inhibition: a) *Bacillus cereus;* b) *Staphylococcus aureus;* c) *Bacillus subtilis;* d) *Staphylococcus albus* (C=Control; SE=Seed extract; ST=Standard)

Effect of Leaf and Seed Extracts on Development of Silkgland: The effect of leaf and seed extracts on the development of silkgland on 1st, 3rd, 5th and 7th day was studied and the data are presented in Table–2 and shown in Fig.3. The green weight of silk gland found to be enhanced on 1st day to 7th day of treatment in both leaf and seed extracts of *T. cucumerina* in comparison to control. After 7th day of the treatment, maximum increased weight was recorded in the green weight of silk gland. Even dry weight has also been found to be enhanced in all the treatments after 1st to 7th day in leaf and seed extracts compared to control. Seed extracts have shown maximum enhanced effect than the leaf extracts of *T. cucumerina*.

From these results, it is found that there is an impact of the methanolic leaf and seed extracts on the development of silk gland. Considerable amount of silk gland weight was also found to be increased in seed extracts in comparison to leaf extracts (Fig. 3).

Table 2: Development of silk gland of silkworm exposed to leaf and seed extracts of *T. cucumerina*

		Control			Treatment with leaf extracts			Treatment with seed extracts		
S.No	Age of silk worm (days)	Green weight of SG (mg)	Dry weight of SG (mg)	Moisture content (%)	Green weight of SG (mg)	Dry weight of SG (mg)	Moisture content (%)	Green weight of SG (mg)	Dry weight of SG (mg)	Moisture content (%)
1	1st	36	6	88.5	41	6	58.2	43	87	84.3
2	3rd	60	7	92.2	66	8	88.6	69	10	87.4
3	5th	205	23	89.5	215	26	90.2	260	30	92.6
4	7th	510	56	89.4	525	60	91.5	540	65	93.2

SG= Silkworm gland

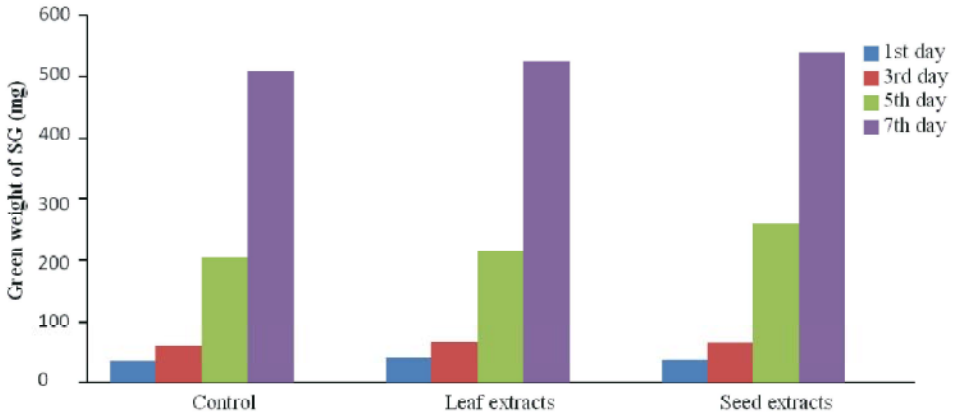

Fig. 1: Development of silk gland of silkworm exposed to leaf and seed extracts of *T. cucumerina*

Effect of Leaf and Seed Extracts on Linear Length of Silk Gland: The results on the effect of methanolic extracts of leaf and seed of *T. cucumerina* on linear length of the silk gland are presented in Table 3 and shown in Figs. 3–4. Linear length of silk gland was found to be increased in all the days after treatment with the leaf and seed extracts of *T. cucumerina* in comparison to control. Maximum gland length of posterior region was observed in seed extracts after 7th day of treatment followed by leaf extracts. It was interesting to note that in all the regions (anterior, middle & posterior) of silk gland, the length has been enhanced with leaf and seed extracts treated than the control (Figs. 3–4).

Table 3: Effect of leaf and seed extracts of *T. cucumerina* on linear length of silk gland

		Control			Treatment with leaf extract			Treatment with seed extract		
S. No.	Age of Silkworm (days)	Length Anterior Region (cm)	Length of Middle Region (cm)	Length of Posterior Region (cm)	Length Anterior Region (cm)	Length of Middle Region (cm)	Length of Posterior Region (cm)	Length Anterior Region (cm)	Length of Middle Region (cm)	Length of Posterior Region (cm)
1	1st	2.2±0.06	1.5±0.02	4.3±0.06	2.3±0.02	1.7±0.05	4.7±0.04	2.2±0.05	1.8±0.05	5.1±0.08
2	3rd	2.4±0.05	4.5±0.02	4.7±0.05	2.5±0.04	4.8±0.07	4.8±0.02	2.5±0.14	5.0±0.04	5.5±0.14
3	5th	3.3±0.03	5.1±0.01	5.6±0.01	4.0±0.01	5.3±0.02	5.7±0.03	3.3±0.06	5.5±0.01	6.1±0.03
4	7th	3.4±0.06	5.3±0.04	8.0±0.01	4.5±0.05	5.7±0.01	8.5±0.12	3.6±0.06	6.4±0.02	8.7±0.08

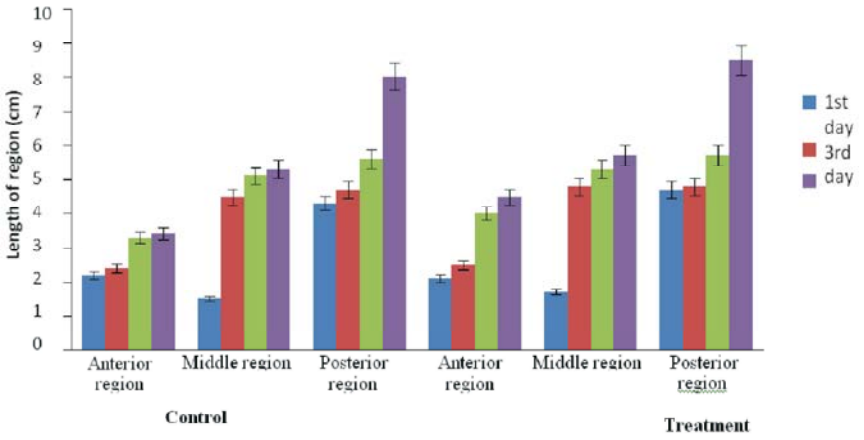

Fig. 2: Linear length of the silk gland exposed to leaf extracts of *T. cucumerina*

Effect of Leaf and Seed Extracts on Commercial Characters of Silk Worm: The results on the effect of leaf and seed extracts of *T. cucumerina* on commercial characters are presented in Table–4. Maximum cocoon weight was observed in seed extracts followed by leaf extracts of T_1 concentration than the control. T_1 extracts have shown enhanced effect of all the commercial characters followed by T_2 and T_3 concentrations (Plates–I–IV). An average pupal weight, shell weight and average silk percentage have also been found to be maximum in all T_1, T_2 & T_3 seed extracts than the leaf extracts. An average filament length was also increased in silk worms fed with T_1, T_2 & T_3 concentrations of leaf and seed extracts. It was recorded that the seed extracts have shown maximum enhancement of all the commercial characters studied (Fig. 4).

Table 4. Effect of leaf and seed extracts of *T. cucumerina* on commercial characters of silk worm

	Leaf Extracts					Seed Extracts				
Treatments	Average cocoon weight (gm)±SE[a]	Average pupal weight (gm) ±SE[a]	Average shell weight (gm) ±SE[a]	Average silk percentage (%)	Average filament length (cm) ±SE[a]	Average cocoon weight (gm)±SE[a]	Average pupal weight (gm) ±SE[a]	Average shell weight (gm) ±SE[a]	Average silk percentage (%)	Average filament length (cm ±Se[a]
T_1	1.94±0.11	1.54±0.08	0.39±0.01	20.31	97±0.17	2.1±0.12	1.6±0.06	0.45±0.01	23.0	100±0.12
T_2	1.83±0.04	1.48±0.06	0.32±0.02	19.02	88±1.19	1.9±0.20	1.5±0.08	0.35±0.02	22.0	95±1.20
T_3	1.74±0.02	1.42±0.03	0.32±0.01	18.65	80±2.03	1.8±0.02	1.45±0.02	0.33±0.01	19.0	85±1.24
C	1.63±0.02	1.38±0.03	0.310±0.01	18.13	69±1.09	1.65±0.04	1.35±0.04	0.28±0.01	17.2	65±1.02

T_1(1:1), T_2(1:2), T_3(1:3), C=Control, Data representing an average of 10 cocoons; ±SE[a]=Mean±Standard Error

Fig. a–f: Effect of leaf extracts of *T. cucumerina* on commercial characters of silk worm
a) Leaf extracts in a petriplate
b) Silkworms feeding on mulberry leaves fortified with leaf extract c) Reared silkworms fed with N, T_1, T_2 & T_3 samples respectively
d) Length of silkgland after treatment with N, T_1, T_2, T_3 samples respectively
e)Cocoons of *B. mori* after treatment(N,T_1,T_2,T_3) (Note the difference in size)
f) Silk weight (gms) after treatment (N, T_1, T_2, T_3)

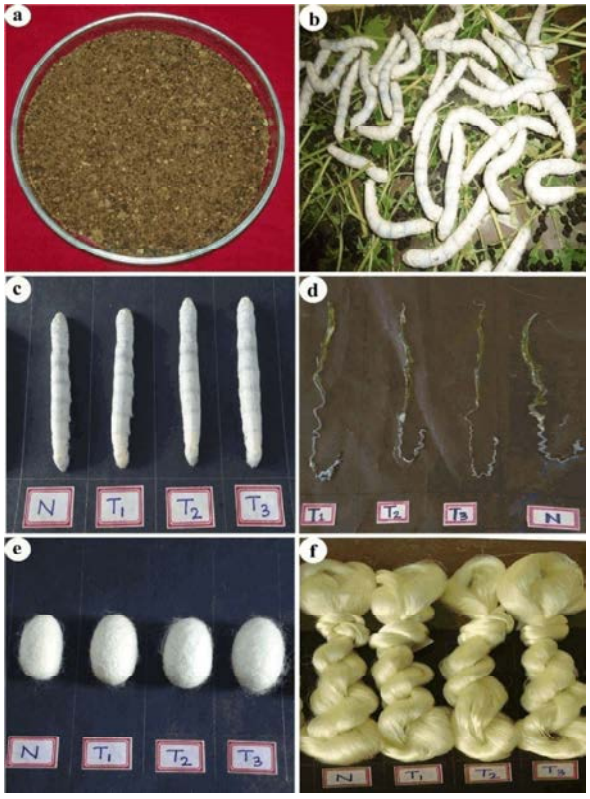

Fig. a–f: Effect of seed extracts of *T. cucumerina* on commercial characters of silk worm
a) Seed extracts in a petriplate
b) Silkworms feeding on mulberry leaves fortified with seed extracts c) Reared silkworms fed with N, T_1, T_2 & T_3 samples
d)Length of silkgland after treatment with N, T_1, T_2, T_3 samples
e)Cocoons of *B. mori* after treatment (N,T_1,T_2,T_3) (Note the difference in size)
f) Silk weight (gms) after treatment (N, T_1,T_2,T_3)

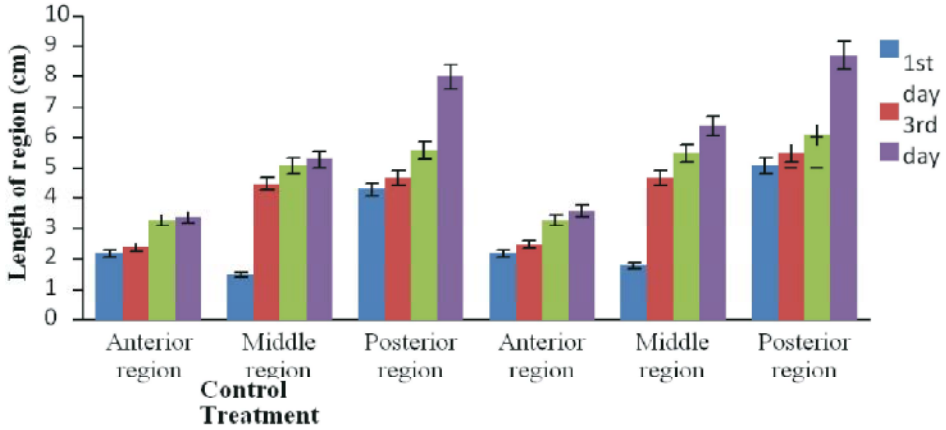

Fig. 3: Linear length of the silk gland exposed to seed extracts of *T. cucumerina*

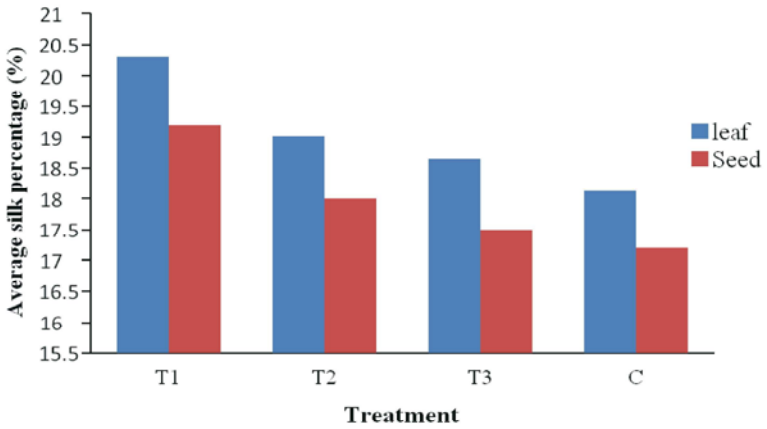

Fig. 4: Effect of leaf and seed extracts of *T. cucumerina* on commercial characters of silk worm

DISCUSSION

In the present investigation the leaf and seed methanolic extracts showed the antibacterial activity against the gram positive and negative bacteria which infect silkworms. Silk worms fed with these extracts have shown the direct influence on development and length of silk gland. According to our observations the gland size affected the silk production in the form of quantity and quality.

The leaf and seed extracts of *T. cucumerina* have shown the enhanced effect on all the commercial characters of silk worm studied than the control. This positive effect of the extracts was not due to one main constituent, but due to the combined action of other chemical compounds present in the extracts [18].

The present investigation revealed the activity of different proportions of seed and leaf extracts of *T. cucumerina* could be an important source of a potent antibacterial medicine for silk worms. These extracts have not only shown the antibacterial activity but also found the enhanced effect on commercial characters of silk worm as observed by Samatha *et al.,* [19]. They have also recorded similar findings in silkworm by using the extracts of stem bark of *Oroxylum indicum* to increase biological characters such as larval, cocoon, pupal and shell weight, shell ratio percentage and length of silk filament. Because these extracts influence the esterase intensity and hence there is an increase in the silk yield. Thus, it can be used as a nutrient supplement to improve silk yield in *B. mori*.

It is evident from our findings that the phytochemicals / bioactive compounds present in the leaf and seed can be used as antimicrobials to cure many diseases in commercially important insect silkworm. Bioassay studies against silkworm showed no ill effects on the silk worm larvae after feeding with the seed and leaf extracts at three different concentrations (T_1, T_2& T_3). The maximum ZI, at T_1 concentration with increase in commercial characters provide a base for use of the plant in the treatment of bacterial diseases in silk worm besides increasing in silk production and thereby helping in discovering a new secondary metabolite, for the industry.

CONCLUSION

Thus, based on our investigations, it can be concluded that these methanolic leaf and seed extracts on the growth and production of the mulberry silk worm, *B. mori* are likely throw light on the possibility of using such extracts as a prophylactic measure during silk worm rearing and also to improve silk production.

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