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Effect of leaf and seed extracts of *T. cucumerina* L on antibacterial activity and economic traits of mulberry silkworm, *Bombyx mori* L

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Abstract
The present work has been undertaken to study the effect of leaf and seed extracts of *Trichosanthes cucumerina* L in the flacherie disease that infect mulberry silkworm larvae. The mulberry leaves fortified with different concentrations of the extract was fed to the worms orally and its influence on IVth instar larvae, silk gland and economic traits were studied. The methanolic extracts showed maximum antibacterial activity. The various concentrations (1:1, 1:2 and 1:3) of methanolic extracts increased the length and weight of silk gland on alternate days in IVth instar. Both extracts of T1 treatment not only showed highest zone of inhibition against all gram positive and gram negative bacteria but also influenced the larval and cocoon characteristics of *Bombyx mori* L Where T3 concentration showed lowest zone of inhibition against the bacteria except against *S. albus*, while T2 recorded moderately low antibacterial activity and cocoon weight (1.94± 0.11g, 2.1± 0.12g), pupal weight (1.54± 0.08g, 1.60±0.06g), shell weight (0.39± 0.01g, 0.450±0.01g), silk percentage of filament (20.31%, 23%) and filament length (8901± 17g, 9000± 0.12) were recorded over the control of both the plant extract of leaf and seed respectively
In the present investigations moderate concentration of *T. cucumerina* extract have showed promoting effect on economical traits of silkworm

Keywords: Mulberry silkworm, plant extracts, zone of inhibition, silk gland and economic traits

1. Introduction
Plants are warehouses of phytochemicals which play a vital role for the development of modern drugs with antibacterial, antifungal, antiviral and anticancer properties, etc. Out of the estimated 2, 50, 000 flowering plant species, only 5000 have been thoroughly studied according to the Natural Product Alert (NAPRACERT) data base.
Host plant suitability of phytophagous insects involves nutritional and non-nutritional factors of the host. The feeding behavior of silkworm is altered in response to the nutritional requirements and dietary composition [15] the plant allelochemicals influence the host selection, feeding behavior and population dynamics [13]. Botanicals have immense ability to influence the metabolic activities of insects [9]. Hence, in the past, attempts were made to fortify mulberry leaves with botanical extracts so as to improve the quality of mulberry leaf and feeding efficiency of silkworm and subsequently the silk production [10, 11].
In the present investigation, the effect of methanolic extracts of *T. cucumerina* on larval growth and commercial parameters have been examined in the hope of finding that this extract doesn't have any negative effect on the larvae and can be used in improving the economical traits of silkworm The extract is prepared from *T. cucumerina* which belongs to family Cucurbitaceae which comprised 130 genera and more than 900 species [16]. The genus *Trichosanthes* is the largest genus of this family.

1.1 Vernacular names
Telugu	-	Adavipotlakaya
Hindi	-	Chachinda
Tamil	-	Pudalankaei
Sanskrit	-	Amritaphala

1.2 Botanical plant description and chemical constituents

It is a wild plant in Southern India and is closely related to *T. angulina*. It is an annual climber with leaves 2-4 inches in diameter, obtuse and cordite. Flowers are unisexual and white in color, the fruit is a berry with white stripes when green and red when ripen, it tastes bitter and contains wide range of medicinal properties.

The chemical constituents are cucurbitacin B and E, sterol, β sistosterol and stigmasterol. It is highly constituted with proteins, fat, fibre, vitamin A and B, total phenol (46.8%) and flavonoid (78%) respectively.

2. Material and Methods

2.1 Plant material

The botanical parts of *T. cucumerina,* collected from Kakatiya University were shade dried, finely powdered and stored in air tight containers for experimental purpose

2.2 Preparation of the plant extracts

The shade dried leaves and seeds were powdered individually and 25g of each was extracted with 150 ml of methanol (80%) for 24 hrs in Soxhlet equipment and filtered through 0.45µm membrane filter. These filtrates were evaporated in a rotary evaporator (55 ^{0}C) under reduced pressure and used as stock. For experimental purpose, fresh extracts of different concentrations of (leaf and seed: water) were prepared on every third day (T1=1:1, T2=1:2, T3=1:3).

2.3 Antibacterial activity

The used bacteria were *Bacillus cereus-KUCC23*, *Bacillus subtilis-KUCC 17*, *Staphylococcus aureus-MTCC 96* and *Staphylococcus-MTCC 96*

2.4 Preparation of bacterial nutrient medium

On the surface of sterile agar plates, standardized 1 to 2x10^{4}cfu/ml 0.5 MC Farland standards were introduced and inoculums were evenly distributed using a sterile glass spreader, using a sterile cork borer 8mm wells were prepared in the plates in which 50µl of extracts of 200mg/ml concentration was introduced and plates were incubated at 37 ^{0}C

2.5 Preparation of test solution for antibacterial activity

A concentration of 250 mg/ml each methanolic extract of leaf and seed of *T. cucumerina* was prepared in DMSO (which doesn't influence the growth of microbes). After 24 hrs, the zone of inhibition was measured with a caliper and 10mg/ml of gentamycin was used as standard. The antibacterial activity results were expressed as mean±SD

2.6 Procedure for treatment of silkworms

Ten DFLs of Bivoltine dihybrid race were purchased from Vijayawada grainage for the study and reared on V1 mulberry variety in the rearing house as recommended [1]. After second moult, the larvae were selected for the treatment and divided into groups, where each group consisted of three replicates with 100 larvae each. The stock solution (100 ml) was diluted into three different concentrations (T1=1:1, T2=1:2, T3=1:3) with help of a atomizer sprayed on required quantity of mulberry. After shade drying, the silkworms were fed with the fortified mulberry leaves. Mulberry leaves sprayed with distilled water served as control. The observations on economic parameters were studied.

2.7 Isolation of silk gland from silkworm larvae

The silkworm larvae were cut opened longitudinally along the mid dorsal line and a pair of silk gland were removed from the late age larvae, washed twice in ice cold insect ringer and kept in separate Eppendorf tubes containing 200µl of 0.01% SDS and later the silk gland was transferred on to the slide to measure its length.

The silk gland was weighed on an electronic balance in chilled state to record the green weight or wet weight of the silk gland, later it was dehydrated for 24 hrs in the hot oven at 100 ^{0}C, which gives the dry weight of the silk gland. The difference in green weight and dry weight was taken as moisture content and its percentage.

2.8 Data Analysis

The data of the parameters were statistically analysed [2]. The statistical analysis of the data was carried out using Student's t-test and the results were considered significant when p ›0.05

3. Results

The effect of methanolic seed and leaf extract of *T. cucumerina* were studied not only against flacherie disease but also for the enhancement of economic traits in mulberry silkworm.

3.1 Antibacterial activity of methanolic extracts of leaf and seed

The results of the antibacterial activity of methanolic extract of the selected plant are shown in Table-1 and Figs1&2. The inhibition zone diameters by the test isolates against *T. cucumerina* was high at T1 concentration in comparison to the standard. The highest antibacterial activity of 10.6 ±0.8mm in *Bacillus subtilis* at T1 concentration of leaf extract and lowest activity of 3.6±0.4mm in *B. cereus* was measured. The leaf extract of *T. cucumerina* exhibited highest activity in the range of 10-7mm against *B. subtilis* and *Staphylococcus aureus* and lowest activity in *B. cereus* at T3 concentration. The leaf extract has shown the highest inhibition zone (10.6mm) against bacteria followed by the seed extract (9.8 mm) at T1 concentration. Leaf and seed extract of this plant showed high inhibitory activity against *B. subtilis* and *S. aureus* at all concentrations when compared to all the other tested bacteria

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

Concentration of seed extract	Leaf Extracts				Seed Extracts			
	Bacillus subtilis **(ZI in mm±SE)^a**	***Bacillus cereus*** **(ZI in mm±SE)^a**	***Staphylococcus albus*** **(ZI in mm±SE)^a**	***Staphylococcus aureus*** **(ZI in mm±SE)^a**	***Bacillus subtilis*** **(ZI in mm±SE)^a**	***Bacillus cereus*** **(ZI in mm±SE)^a**	***Staphylococcus 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

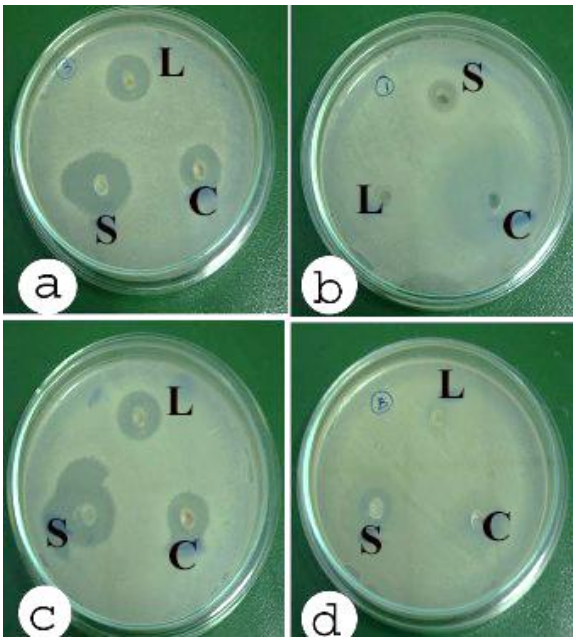

Plate I: Fig a-d: Antibacterial activity of leaf extracts of *T. cucumerina* showing zone of Inhibition (in mm): **a)** *Bacillus cereus* **b)** *Staphylococcus aureus* **c)** *Bacillus subtilis* **d)** *Staphylococcus albus*(C=Control; L=Leaf extract; S=Standard)

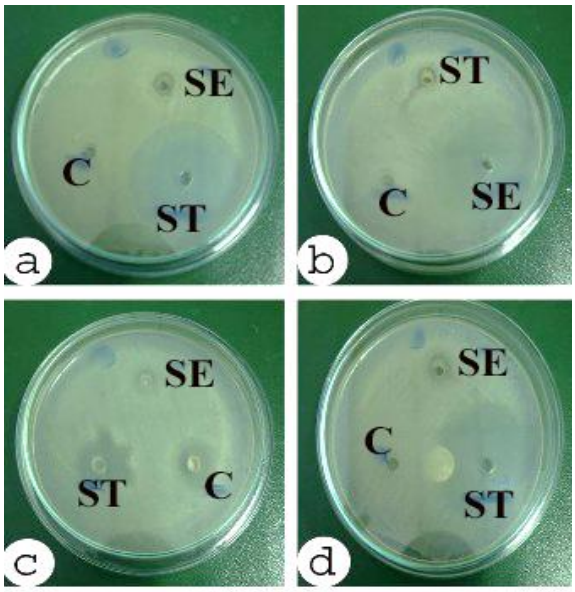

Plate II: Fig a-d: Antibacterial activity of seed extracts of *T. cucumerina* showing zone of Inhibition: **a)** *Bacillus cereus* b) *Staphylococcus aureus* **c)** *Bacillus subtilis* **d)** *Staphylococcus albus*(C=Control; SE=Seed extract; ST=Standard)

3.2 Effect of leaf and seed extracts on silk gland

The effect of leaf and seed extract on silk gland was studied on 1st,3rd,5th and 7th day and the data was presented in Table -2 and Fig. 3.The green weights of silk gland treated with seed extracts increased from 1st to 7th day over the leaf extract treated batches and control.

The values obtained with seed extracts and control on the 7th day for the green weight, dry weight and moisture content are 540 mg, 65mg and 93.2% and 510mg, 56mg and 89.4% respectively.

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

S. No	Age of silk worm (days)	Control			Treatment with leaf extracts			Treatment with seed extracts		
		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 S.G (mg)	Dry weight of S.G (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

Note: Data is mean of three readings

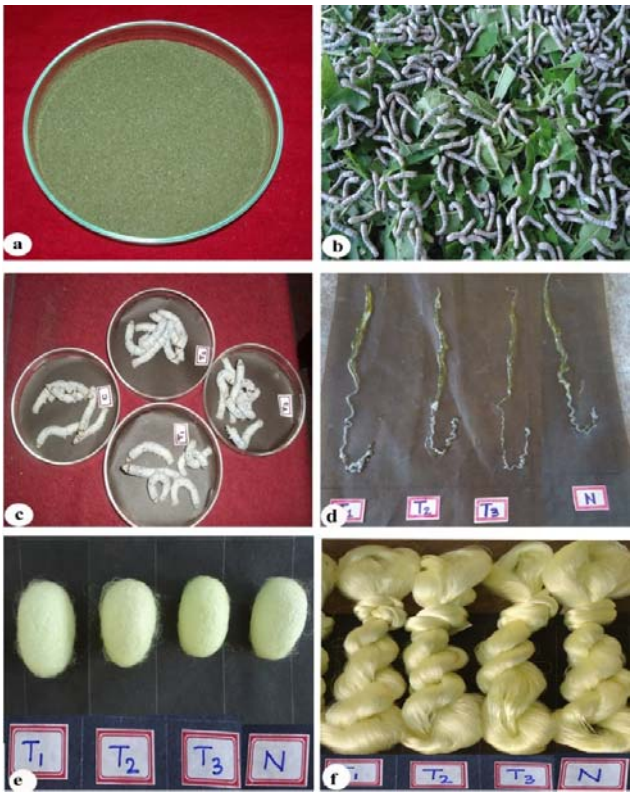

Plate III: Fig a-f: Effect of leaf extracts of *T. cucumerina* on commercial characters of silk worm **a)** Leaf extracts in a Petri plate **b)** Silkworms fed with mulberry leaves fortified with leaf extract **c)** Fed silkworms with T_1, T_2, T_3&N=control samples respectively **d)** Length of the silk gland after various treatments (T_1, T_2, T_3&N=control) **e)** Cocoons of *Bombyx mori* (T_1, T_2, T_3&N=control)(Note the difference in size) **f)** Skeins samples (g) (T_1, T_2, T_3&N=control)

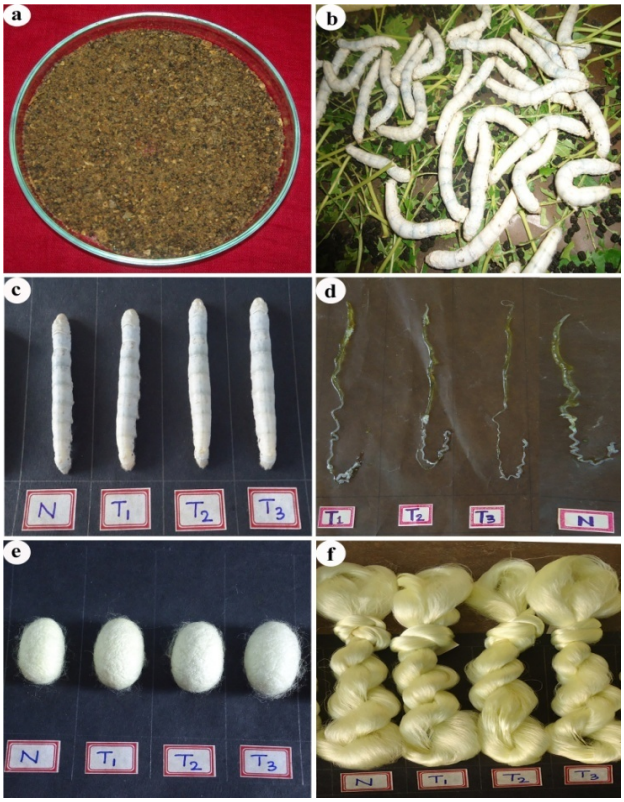

Plate IV: Fig a-f: Effect of seed extracts of *T. cucumerina* on commercial characters of silk worm **a)** Seed extracts in a Petri plate **b)** Silkworms fed with mulberry leaves fortified with seed extract **c)** Fed silkworms with N=control, T_1, T_2 & T_3 samples **d)** Length of the silk gland after various treatments (N=control, T_1, T_2, T_3) **e)** Cocoons of *Bombyx mori* (N=control, T_1,T_2,T_3)(Note the difference in size) **f)** Skeins samples (g) (N=control, T_1,T_2,&T_3)

3.3 Linear length of the silk gland in the treated worms

The observations showed linear development of the silk gland of the worms fed with leaves fortified with *T. cucumerina* are presented in Table 3 and shown in Figs3-4. The linear length of silk gland increased after treatment in comparison to control. Maximum silk gland length was observed in seed extract batches when compared to leaf extract batches

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

S. No	Age of Silkworm (days)	Control			Treatment with leaf extracts			Treatment with seed extracts		
		Length of Anterior Region (cm)	Length of Middle Region (cm)	Length of Posterior Region (cm)	Length of Anterior Region (cm)	Length of Middle Region (cm)	Length of Posterior Region (cm)	Length of 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

3.4 Effect of leaf and seed extracts of *T. cucumerina* on commercial characters of silkworm

The results of the plant extract's effect on economic traits are presented in Table-4, significant differences existed among the *T. cucumerina* extracts treated batches when orally fed to the worms on economic traits of silkworm at different concentrations, seed extract of *T. cucumerina* at T1 concentration recorded maximum cocoon weight (1.94g), pupal weight (1.54 g), shell weight (0.39g), silk ratio percentage (20.31) and filament length (720 m), while the leaf extract recorded high cocoon weight, pupal weight and shell weight silk ratio percentage and filament length than control. The above plant extracts at T3 concentration recorded low economic parameters than control. It was recorded that the seed extract showed maximum enhancement of all economic characters studied in comparison to leaf treatments and control (Fig. 4)

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

Treatments	Leaf Extracts					Seed Extracts				
	Average cocoon weight (gm)±SE[a]	Average pupal weight (gm) ±SE[a]	Average shell weight (gm) ±SE[a]	Average silk percentage (%)	Average filament length (m) ±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 (m) ±SE[a]
T_1	1.94±0.11	1.54±0.08	0.39±0.01	20.31	717±0.17	2.1±0.12	1.6±0.06	0.45±0.01	23.0	720±0.12
T_2	1.83±0.04	1.48±0.06	0.32±0.02	19.02	688±1.19	1.9±0.20	1.5±0.08	0.35±0.02	22.0	695±1.20
T_3	1.74±0.02	1.42±0.03	0.32±0.01	18.65	680±2.03	1.8±0.02	1.45±0.02	0.33±0.01	19.0	685±1.24
C	1.63±0.02	1.38±0.03	0.310±0.01	18.13	669±1.09	1.65±0.04	1.35±0.04	0.28±0.01	17.2	665±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

4. Discussion

Plant extracts at different concentrations showed tendency not only in improving many of the economic characters but also showed antibacterial activity against the bacteria that infected the silkworms. Bioassay studies against silkworm showed no ill effects after feeding with *T. cucumerina.* Plant extracts fed larvae enhanced the larval and economic parameters of the silkworm [3-6] and also helped in the control of flacherie disease. Similar findings in silkworm by using the extracts of stem bark of *O. indicum* to enhance commercial characters in silkworm have also been recorded. The leaf and seed extracts have shown enhanced effect on the economic traits like cocoon, shell and pupal weight which are major traits which influence the silk production and quality. These reports clearly show that the response of silkworm in terms of improvement in economic traits varies with compounds used, silkworm races and geographical region [8]. This positive effect of the extract was not due to one main constituents but due to the combination of other chemical compounds present in it [7]. The maximum inhibition zone at T1 concentration with increase in economic parameters provides a base for use of this plant in control of flacherie disease besides the enhancement of economic parameters in silkworms and thereby helping in discovering a new secondary metabolite for the industry.

5. Conclusion

Thus, based on the investigation it can be concluded that these methanolic extracts on growth and production of the mulberry silkworm are likely to throw light on the possibility of using these extracts not only as a prophylactic measure during silkworm rearing but also for enhancing the commercial characters of silkworm

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7. Reference

1. Datta RK, Basavaraja MK, Mano Y. Manual on bivoltine rearing race maintenance and multiplication. Central Sericulture Research and Training Institute, Mysore. 1996, 65.
2. Pillai SK, Simha HC. In statistical methods, for biological workers, Ramprasad and Sons, Agra, 1968.
3. Sujatha Kuntamalla, Sampath Akula. Clove oil for the control of flacherie disease and its effect on economic traits of silkworm, *Bombyx mori*. Int J Pharm. Bio science. 2015; 6:1311-1323
4. Sampath A, Ramesh Babu M, Sujatha K, Jaikishan Singh RS, Digamber Rao B. Beneficial effect of cyanobacteria

Anabaena variabilis on quantitative traits of Eri silkworm *Samia cynthia ricini*. Asian Journal of Agricultural Science. 2013; 5(3):36-39
5. Kuntamalla Sujatha, Sammaiah Ch. Influence of major constituents in of *Curucuma longa* against bacterial disease and on larval and economic traits in silkworm *B. mori*. J Exp Zoo. India. 2007; 10(2):497-500.
6. Samatha T, Sampath A, Sujatha K, Nanna RS. Effect of stem bark extract of *oroxylum indicum*; An ethno medical forest tree on silk production of *B. mori*, Int. J Pharm Sci. Res. 2014; 5(2):568-571.
7. Bai D. Traditional chines malarial; A respect and prospect. Plant Med. 1990, 50-502
8. Nair KS, Nair JS, Trivedy K, Vijayan V. Influence of Bakuchoil. JH Analogue from Bemchi (Psoraleacorylifolia) on silk production in silkworm Journal of applied science and Environmental Management. 2003; 7(2):31-38
9. Murakoshi S, Chang CF, Kamikado T, Tamuras. Effect of the extract from leaves of various plants on the growth of silkworm, J Applied Entomol and Zool. 1975; 19(3):208-213
10. Krishnaprasad NK, Sanappa B, Reshma, Keshava Prasad PK. Effect of solanaceous leaf extracts on growth development and cocoon traits of PM X NB4D2 Silkworm (*B. mori*) Bull. Indian Acad Seic. 2001; 5:42-47.
11. Jayapaul C, Padmalatha C, Singh AJAR, Murugesan AG, Dhasarathan P. Effect of plant extract on nutritional efficiency in mulberry silkworm, *Bombyx mori*, Indian J Seric. 2003; 42(2):123-131
12. Morohoshi S. Development Physiology of silkworm (I) in the silkworm- An important laboratory tool, Y. Tazima (Ed), 1976.
13. Barabosa P, Schultz TC. (EDS).Insect out breaks. Academic press, Newyork. 1987, 66-68
14. Murugan K, Ancy George SR. Feeding and nutritional influence on the growth and reproduction of *Daphnis nerii* Linn (Lepidoptera Sphingidae). J Insect Physiol. 1992; 38:961-968
15. Slansky F Jr. Allelochemical nutrient interactions in herbivore nutritional ecology. In Herbivores their interactions with secondary plant Metabolites. Volume II Evolutionary and ecological process (Rosenthal (G.A ed). Academic press, Sandiego. 1992, 135-174.
16. Chakravarhy HC. Fascicles of flora of India fascicles H. Cucurbitaceae, Botanical survey of India, Howrah, 1982, 73