Identification of Certain Chemicals and Medicinal plants having potential to suppress the Flacherie disease in Silkworm Bombyx mori L.

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INTRODUCTION

In India, the extant of cocoon crop loss due to the silkworm diseases is nearly 40% (Sahay *et al.*, 2000). As there are no curative measures in silkworm rearing for the control of silkworm disease, different preventive methods using chemical disinfectants are practiced (Singh *et al.*, 2002, 2005). Thangavelu *et al.* (1995) used various chemicals as disinfectants for the control of different diseases in tasar silkworm. Bhattacharaya *et al.* (1995), Datta *et al.* (1998) and Samson *et al.*, (1998) Patil and Shardamma (1999) used chemicals based body and rearing seat disinfectants like Labex, Sanjeevani, Vijetha, Resham Jyoti for management of diseases in mulberry silkworm *Bombyx mori*. Phenolic compound (Henga, 1977), Sodium hypochlorite and formalin (Vail *et al*, 1968) and formalin (Ignoff and Garcia, 1968) against several pathogens. Various disinfectants viz. formalin (Kagawa, 1980), Asiphore (Venkata Reddy *et al*, 1990), Chlorinated lime and hydrochloric acid (Miyajima, 1979), Chlorine dioxide (Balavenkatasubbaiah *et al.*, 1999) were tested against silkworm pathogens. Baig *et al.*, (1989) formulated a mixture of paraformaldehyde, benzoic acid and lime as a bed disinfectant against Nuclear polyhedrosis of mulberry silkworm. Bansal *et al.* (1996) tested Asiphore and sodium hypochlorit against virosis in tasar silkworm. T.K.O and Jeevan Suraksha are developed by CTR&TI is only confined to containment of bacteriosis and virosis diseases.

Therefore the bacterial pathogens causing flacherie disease in silkworm *Bombyx mori* L. subjected for the in vitro inactivation studies with four chemicals namely Sodium Silicate Pentahydrate, Tri-sodium orthophosphate, Benzalkonium chloride and Didecyldimethylammonium chloride, which are known for the germicidal activity. Results revealed that, 1.5 to 3% of Tri-Sodium Orthophosphate is capable to dissolution of suppression of bacterial at two to five minutes sterilization time. 1.0% to 5.0% of Benzalkonium chloride and Didecyldimethylammonium chloride have given best results in suppression of bacterial pathogens from two to five minutes sterilization time. Based on in vitro studies, these chemicals can be utilized for making disinfectant for the containment of various diseases of silkworm after confirmation with in vivo studies. Hence, an attempt was made to test different chemicals against bacterial disease causing pathogens of silkworm to find out efficacy. Based on in vitro studies, these chemicals can be utilized for making broad spectrum disinfectant for the containment of various diseases of silkworm after confirmation with in vivo studies.

Materials and methods:

All the experiments required of the study on In vitro inactivation studies of certain antimicrobial chemicals against bacterial disease causing (Bacillus thuringiensis) conducted in, Dept of Sericulture, Sri Krishnadevaraya University, Anantapur, Silkworms were reared as per the package practices up to the end

of the experiment (Krishnaswami *et al.*, 1973).In this experiment 5th instar larvae are taken to study targeted research aspects.

Preparation of chemicals concentrations:

1.0, 1.5, 2.0, 2.5, and 3.0% solutions of Sodium Silicate Pentahydrate, Tri-sodium orthophosphate, Benzalkonium chloride and Didecyldimethylammonium chloride were prepared by dissolving 1.0, 1.5, 2.0, 2.5, and 3.0 g of the same in 100 ml of sterile distilled water, respectively.

Isolation and purification of Bactria:

One ml of the suspension of pathogen of bacteria having concentration of 1 x10^{-7} cells/ml added to the1.0 ml of the above mentioned concentrations of disinfectants for different durations viz. 1,2,3,4, and 5 minutes at room temperature (25 ± 1 °C) and added in to sterile Petri plates. There after melted and cooled (42-45°C) agar medium in bacteria was poured and mixed thoroughly by rotating the plates which were then allowed to solidify. Then the plates were incubated at 37°C for 24 to 48 hrs and the observations were recorded and analyzed.

Results:

Sodium Silicate Pentahydrate, Tri-sodium orthophosphate, Benzalkonium chloride and Didecyldimethylammonium chloride were tested for their efficacy against disease causing pathogens of silkworm bacteria (Bacillus thuringiensis).

Growth of different Bacteria in in vitro after sterilization with Sodium Silicate Pentahydrate:

1% to 2.5% concentration of Sodium Silicate Pentahydrate has not shown impact on growth of Bacillus at 1 to 5 minutes sterilization time. These bacteria have grown luxuriantly just like control. But, 4 and 5 minutes sterilization time of 3%concentration have shown partial growth of Bacillus.

Tri-sodium orthophosphate:

1% concentration of Tri-sodium orthophosphate has not shown great impact on growth of Bacillus at 1 to 3 minutes sterilization time. But, 1% concentration 4 and 5 minutes sterilization time have shown partial growth of Bacillus. At the 1.5% concentration, partial growth of Bacillus was observed at 1 minute sterilization time whereas from 2-5 minutes sterilization time no growth was observed. From the concentration 2 to 3% no growth of bacteria was observed at any sterilization timings.

Benzalkonium chloride and Didecyldimethyl ammonium chloride:

1% to 3% concentration of Benzalkonium chloride and Didecyldimethyl ammonium chloride has shown no growth of Bacillus at 1 to 5 minutes sterilization time expect 1% concentration 1 minute sterilization time which have shown partial growth.

***Table1*. Growth of *Bacteria* in in vitro conditions, after sterilization with different concentrations of chemicals at different duration.**

Disinfectant	***Conc %***	***Sterilizationn Duration: min***				
		1	***2***	***3***	***4***	***5***
Sodium Silicate Penta hydrate	***1.0***	+	+	+	+	+
	1.5	+	+	+	+	+

	2.0	+	+	+	+	+
	2.5	+	+	+	+	+
	3.0	+	+	+	±	±
Tri-sodium orthophosphate	***1.0***	+	+	+	±	±
	1.5	±	-	-	-	-
	2.0	-	-	-	-	-
	2.5	-	-	-	-	-
	3.0	-	-	-	-	-
Benzalkonium chloride	***1.0***	±	-	-	-	-
	1.5	-	-	-	-	-
	2.0	-	-	-	-	-
	2.5	-	-	-	-	-
	3.0	-	-	-	-	-
Didecyldimethyl ammonium chloride	***1.0***	±	-	-	-	-
	1.5	-	-	-	-	-
	2.0	-	-	-	-	-
	2.5	-	-	-	-	-
	3.0	-	-	-	-	-

DISCUSSION

The results of the study indicated that, from the concentration 1.5% to 3%concentration of Tri-sodium orthophosphate has shown great impact on growth of Bacillus at 1 to 5 minutes sterilization time. 1% to 2.5% concentration of Sodium Silicate Pentahydrate has not shown impact on growth of Bacillus at 1 to 5 minutes sterilization time.

1% to 3% concentration of Benzalkonium chloride and Didecyldimethyl ammonium chloride has shown no growth of bacteria at 1 to 5 minutes sterilization time that means these two chemicals are very good ion suppression of the Bacillus. Similar studies were conducted by the some of the researchers against various pathogens of silkworm and with the various chemicals. Anonymous, 1975 worked with the Formaldehyde and stated that Formaldehyde acts as a reducing agent by deoxidizing the pathogens and kills them, whereas bleaching powder releases nascent oxygen that has strong oxidizing action on the pathogens, chlorine changes the cell membrane to allow diffusion of cell contents and the alkaline calcium has strong germicidal action (Anonymous, 1975). Iwashita and Zhou (1988) reported that polyhedral bodies were dissolved quickly when dipped in saturated solution of calcium hydroxide and virions were inactivated. Similar types of results have been observed in the present study. Balavenkatasubbaiah *et al.* (1994) observed that in slaked lime solution treatment, the polyhedral bodies of BmCPV of *Bombyx mori* were dissolved and inactivated.

The effective chemicals in the present study are to be subjected to the in vivo studies for the confirmation of results. At final these tested chemicals may be useful for the formulation of broad spectrum disinfectant for the containment of various diseases of silkworm *Bombyx mori* L.

Studies of certain antimicrobial botanicals against bacterial disease causing *(Bacillus thuringiensis)* pathogens

Introduction:

The control of infectious diseases is seriously threatened by the continuous increase in the number of microorganisms that are resistant to the chemical antimicrobial drugs (Jazani *et al.*, 2010; Gomah Nenaah, E and Essam Ahmed, M., 2011).The chemical based disinfectants and drug formulations used for prevention/control of this disease are not economic, eco-friendly and also have many limitations in silkworm rearings. Because of this reason disinfectants/drug formulations are ineffective to control this disease at field level. Chemical disinfectants and antibiotics have been used for managing the diseases in silkworm (Kagawa, 1980; Reddy *et al.*, 1990). In view of high cost of chemicals and antibiotics and their hazardous consequences, plant extracts has been on the top priority for control of diseases (Jespers and de Waard, 1993; Kumar *et al.*, 1999). Recently there has been a concerted effort to promote the use of botanicals as possible alternatives to treat infectious diseases (Mohsenzadeh, 2007; Jazani *et al.*, 2009; Chanda *et al.*, 2011). These natural products were found to possess promising antimicrobial activities when applied alone or in combination with conventional antimicrobial drugs (Jazani *et al.*, 2007). Kumar *et al.* (1998) and Manimegalai *et al.* (2000) used plant products and succeeded to control grasserie disease (caused by nuclear polyhedrosis virus) in mulberry silkworm, Bombyx mori. Cellular responses are direct interactions between circulatory haemocytes and invading non self materials. Insects shows defense response through cellular and humoral components (Dunn, 1986, Gupta, 1986).

Effects of various pathogens have been reported in the insects (Horohov and Dunn, 1982; Lea, 1985) however, not much work has been published so far on pathological aspects of flacherie in silkworm. Especially the influence of phytoextracts on haematological defense in the insects infected with flacherie is not available. In the present study, the efficacy of the certain medicinal plants and the influence of top three phytoextracts (having antibacterial properties) on the haemocyte mediated response was reported through study of total hemocyte count and estimation of total protein.

Among the diseases of silkworm Bombyx mori L. Flacherie caused by *Bacillus thuringiensis* is highly contagious and more prevalent. Thirteen anti bacterial plants were used to test their efficacy against flacherie disease in silkworm. The aqueous extracts of these plants in different concentrations were used for containment of bacterial infected silkworm and mortality was recorded. The influence of best three phytoextracts which have shown good results in suppressing flacherie, were subjected for the study of cellular and biochemical changes. 2% aqueous extracts of *Aloe barbedensis* (AKP 3), *P. corylifolia* (AKP13) and *Bougainvillea spectabilis* (AKP 9) were found more effective in suppressing the flacherie and reduced the mortality due to bacterial infection of 66.17%, 64.47% and 57.19%, respectively. The total haemocyte count increased up to 6th day of post inoculation in phytoextract treated batches, while in the inoculated control the increase was within 3 days indicating the positive haemocyte mediated response in silkworm treated with phytoextract. The hemolymph protein in *Aloe barbedensis* treated silkworm (35.27 mg/ml) was significantly higher than inoculated control (20.25 mg/ml). The gradual increase of total hemolymph proteins from 1st day (16.31 mg/ml) to 8th day (33.73 mg/ml) was observed in healthy control where as in inoculated control increasing trend was observed from day 1 (16.26 mg/ml) to day 3 (24.22 mg/ml) there after decreasing trend was observed and finally reached to 20.25 mg/ml (8th day). The plant extract of *Aloe barbedensis* (AKP 3) is more effective in suppressing flacherie, based on the results of mortality reduction against flacherie cellular and biochemical changes.

Materials and Methods;

Thirteen numbers of medicinal plants known for their antibiotic properties were selected and tested for their efficacy against flacherie in silkworm *Bombyx mori* L. Medicinal plants available locally were collected for use in the experiment and coded as AKP1 to AKP 13. Aqueous extracts of plant/plant parts were prepared by grinding 50 g of clean and washed plant materials (leaf/bulb/rhizome) in a little water, filtered through

muslin cloth, made the volume 100 ml and centrifuged at 3000 rpm for 15 min. Final volume was made to 100 ml with distilled water to bring the concentration of the stock to 50%. The stocks were further diluted to desired concentration with distilled water before use.

Inoculation of Bacillus and treatment of larvae with plants extracts: Bacteria isolated from diseased worms was purified and used as inoculums and same is evenly smeared on to Mulberry leaves, air dried and fed to 2nd instar larva ,24 hrs after moult. After 6 hrs of bacterial inoculation and once in 3rd, 4th and 5th instar larvae were allowed to feed on mulberry leaves treated with 1.0 and 2.0% plants extract. Three replications with 100 silkworm larvae each and fourth replication with 50 silkworm larvae (For cellular and biochemical estimation) were maintained separately for each treatment. Both treated and inoculated control batches were reared in two rearings (July – August and September – October) in standard conditions of Silkworm rearing Silkworms were reared as per the package practices up to the end of the experiment (Krishnaswami *et al.,* 1973). The observations were made on development of diseases symptoms and larval mortality. The dead larvae in different treatments during rearing were examined microscopically for presence of concerned pathogen.

Estimation of haemocytes count:

Every day total hemocyte counts (THC) in the hemolymph of all treated and control batches was determined following the method described by Tauber and Yeager, (1934) and (1935) using haemocytometer. The THC per mm^3 of haemolymph was estimated according to the formula suggested by Jonesh (1962). Every day from 1st to 8th day 6 larvae were collected from fourth replication of treated, inoculated and healthy control batches. Hemolymph from all the 6 larvae was collected in to two eppendorf tubes (3 larval hemolymph/tube) on ice and counted total hemocyte count; remaining hemolymph was utilized for estimation of total protein.

Estimation of total protein: The total protein content in hemolymph was estimated by the method of Lowry *et al.* (1951).

Results and Discussion

Efficacy of the medicinal plants

Results of larva mortality and percent of reduction in flacherie in silkworm, *Bombyx mori L.* rearing after inoculation with spores of *Bacillus thuringiensis* and treatment with plant extracts are presented in table 1. Two % aqueous extract of AKP 3 with different concentrations was observed more effective where lowest larva mortality due to flacherie was recorded 32.25 and 28.04% during 1st and 2nd crop rearing, respectively. Comparatively higher larva mortality (86.22% and 85.12%) was observed with the 1% treatment of AKP 4 followed with 2% treatment of AKP 4 (82.33% and 86.66%) and 1% treatment of AKP 7 (82.66% and 80.66%) during 1st and 2nd crops, respectively. In inoculated control (infected with flacherie) larva mortality was 87.00% and 91.66 during 1st and 2nd crops, respectively.

Pooled analysis of data revealed maximum reduction in flacherie 66.17% in the 2% treatments of AKP 3 followed by AKP 13 (64.47%), AKP 9 (57.19%) and AKP 1 (54.15%). Minimum reduction in flacherie was with the 1% treatment of AKP 4 (4.02%).

The aqueous extract of plants AKP 3, AKP 13 and AKP 9 showed encouraging results in reduction of bacterial infection in silkworm larvae rearing. Cocoon parameters like cocoons harvested (51.00%), single cocoon weight(13.57%), single shell weight(1.540g) and silk ratio percent (11.25%) were observed maximum in 2% aqueous extract AKP 3 followed by 1% extract of AKP 3, 2% extract of AKP 13, 1% extract AKP 13 and minimum values were observed in case of control batch (Table 2). Plant extract of all treated batches were on par with the control which indicated that these plant extracts have no adverse effect on the silkworm larvae.

In the present study all the plant extracts tested were found effective in reducing the flacherie. Plant extracts have also shown significant difference with each other in reduction of flacherie in silkworm *Bombyx mori L*. The treatments of aqueous extract of plant AKP 3 (2.00% conc.) was more effective and reduced the bacterial infection to the tune of 66.17% in rearing. Similar studies were made by Manoharraj (1994) and Mallika (1997) where they observed 72.46 and 79.50% reduction in grasserie (caused by nuclear polyhedrosis virus) in mulberry silkworm, *Bombyx mori* by application of leaf extract of *Psoralea coryleifolia*, respectively. Sivaprakasham (1994) reported that the aqueous leaf extract of P. coryleifolia was better than gentamycin or calcium hydroxide in reducing the larval mortality due to grasserie. Manimegalai *et al.* (2000) observed 63% reduction in grasserie disease in B. mori with the application of turmeric and chalk powder. However, plant extracts tested in the present investigation have not been reported earlier against the bacterial disease in silkworm *Bomyx mori* L

Table 1. Silkworm mortality and reduction of Bacterial infection after *Bacillus* inoculation and treatment with plant extracts.

Plants	Concentration	Mortality due to Bacillus			Reduction of bacterial infection from control (%)		
		1st Crop	2nd Crop	Pooled	1st Crop	2nd Crop	Pooled
AKP 1	1	35.67	53.63	44.65	59.00	41.49	50.25
	2	32.00	50.33	41.17	63.22	45.09	54.15
AKP 2	1	78.00	70.33	74.17	10.34	23.27	16.81
	2	74.33	72.66	73.50	14.56	20.73	17.65
AKP 3	1	40.66	47.00	43.83	53.26	48.72	50.99
	2	32.25	28.04	30.15	62.93	69.41	66.17
AKP 4	1	86.22	85.12	85.67	0.90	7.14	4.02
	2	82.33	86.66	84.50	5.37	5.45	5.41
AKP 5	1	42.33	55.00	48.67	51.34	40.00	45.67
	2	37.67	51.67	44.67	56.70	43.63	50.16
AKP 6	1	36.82	52.61	44.72	57.68	42.60	50.14
	2	33.04	50.12	41.58	62.02	45.32	53.67
AKP 7	1	82.66	80.66	81.66	4.99	12.00	8.49
	2	80.00	78.00	79.00	8.05	14.90	11.47
AKP 8	1	80.00	72.00	76.00	8.05	21.45	14.75
	2	77.66	74.33	76.00	10.74	18.91	14.82
AKP 9	1	63.33	56.66	60.00	27.21	38.18	32.70
	2	35.23	41.37	38.30	59.51	54.87	57.19
AKP 10	1	39.18	46.18	42.68	54.97	49.62	52.29
	2	41.67	53.33	47.50	52.10	41.82	46.96
AKP 11	1	42.33	55.33	48.83	51.34	39.64	45.49
	2	40.00	50.66	45.33	54.02	44.73	49.38
AKP 12	1	40.00	48.66	44.33	54.02	46.91	50.47
	2	39.33	46.00	42.67	54.79	49.81	52.30

AKP 13	1	40.00	46.33	43.17	54.02	49.45	51.74
	2	28.50	35.10	31.80	67.24	61.71	64.47
Inoculated control		87.00	91.66	89.33			

Table 2: Effect of plant extracts [Aloe barbedensis (AKP 3), Psoralea corylifolia (AKP13)

Table 2: Effect of plant extracts [Aloe barbedensis (AKP 3), Psoralea corylifolia (AKP13)

Treatment	Conc. %	Cocoons harvested (%)	Cocoon wt (g)	Shell wt (g)	SR %
AKP 3	1	65.80	12.98	1.490	10.87
	2	63.00	13.57	1.540	11.25
AKP 13	1	57.84	12.30	1.370	10.33
	2	53.54	14.04	1.530	10.90
AKP 9	1	54.96	12.77	1.420	10.37
	2	50.03	13.58	1.320	10.49
Control		40.37	12.49	1.410	11.00

Estimation of total hemocyte count:

The data on the effect of aqueous extracts of *Aloe barbedensis* (AKP 3), *P. corylifolia* (AKP13) and *Bougainvillea spectabilis* (AKP 9) on haemocyte count in flacherie infected worms is presented in table 3. In all the treatment sets and in the normal control the total haemocyte count increased significantly from 1st day to 6th days and decreased on 7th day. In AKP 3 batch the total haemocyte counts was 12650/mm^3 of haemolymph and increased to 15432/mm^3 by 6th day. On 7th day total haemocyte counts were 14903/mm^3. On 8th day the counts was increased up to 15625/mm^3. The trend was same in all the treatments and in normal control. While in inoculated control the counts were increased after inoculation with CPV up to 2nd day of infection. Then there was a decrease for a period ranging from 3-8 days. In normal control the total haemocyte counts was significantly low as compared to the treatment because the increase may represent the defense response of silkworm, *Bombyx mori* against the invading pathogen.

The observed data agreed with the earlier workers as they investigated that once entomophagpus fungi have penetrated in the host integument and gained access the nutrient-rich haemocoel, they are confronted with humoral and or cellular defenses. As humoral response, the phenoloxidase system will be activated to induce the phagocytic process and melanization which works as toxin to invading microorganism. The cellular responses to infection have been worked out in many insect by earlier workers (Horohov and Dunn, 1983). Similar type of result also obtained when the effect of systematic fungicide was studied on the total haemocyte count in *B. bassiana* infected silkworm, *Bombyx mori*. Haemocytes are extremely efficient in removing pathogens by accomplishing a series of reactions designated as phagocytosis, nodule formation or encapsulation. The observed data agreed well with the earlier investigation that their number may increase (Balavenkatasubbaiah *et al.*, 2001; Al-Attar, 2010) and decrease to counter foreign body when infected. Recently stress has been induced to tasar silkworm to study its impact on Haemocytes count (Pandey *et al.*, 2010). On the basis of the above findings of the earlier workers it evident that bacteria induce the defence

Table 3. Effect of aqueous extracts of *Aloe barbedensis* (AKP 3), *P. corylifolia*(AKP13) and *Bougainvillea spectabilis* (AKP 9) on the total hemocyte count in bacterial infected silkworm *Bombyx mori* L.

Treatment	Days post inoculation							
	1	2	3	4	5	6	7	8
AKP 3	12650	14266	14810	15266	15407	15432	14903	15625
AKP 13	12750	14305	15000	15530	15833	15903	14856	15457
AKP 9	12500	14236	14607	15111	15527	15543	14230	15008
Inoculated control	12700	14480	14350	12671	9851	6203	5009	3812
Normal control	12333	12455	12782	13124	13690	13702	12480	13713
S.E. ±	132.56	141.21	125.07	106.32	156.78	182.22	191.06	190.23
C. D. at 5%	138.08	151.26	177.12	125.28	256.27	308.15	289.05	331.47

response through multiplication of haemocytes as is indicated by the increase in total haemocyte counts of the haemolymph of the worms. When the treatment was given with aqueous solution of plants having antibiotic activity is suppressing the multiplication of the pathogen and boosting the immunity

level which may be the result of the high total haemocyte count than the healthy control.

Estimation of total protein content in hemolymph

The data on the effect of aqueous extracts of *Aloe barbedensis* (AKP 3), *P. corylifolia* (AKP13) and *Bougainvillea spectabilis* (AKP 9) on protein content in flacherie infected worms is presented in table 4.

The hemolymph protein in aqueous extracts of *Aloe barbedensis* (AKP 3), *P. corylifolia* (AKP13) and *Bougainvillea spectabilis* (AKP 9) silkworms increased gradually from day 1 to day 7 and decreased on day 8. In especially *Aloe barbedensis* (AKP 3) treatment the total protein increased from 16.17 mg/ml to 36.03 mg/ml and reached to 35.27 mg/ml at the end of day 8. In inoculated control, the total hemolymph proteins have shown increasing trend from 1st (16.26 mg/ml) to 3rd day (24.22 mg/ml) and decreasing trend from 4th day onwards and reached 20.25 mg/ml by 8th day from the inoculation. In the healthy control study increase was observed from day 1 to day 8 and reached from 16.31 to 33.73 mg/ml.

Quantitative and qualitiative changes in protein profile of various tissues of tropical tasar silkworm, *Antherea mylitta* D was recently studied (Kumar *et al.,* 2011). The results indicated that changes occur in the hemolymph protein, during the course of AmCPV infection. The difference in total hemolymph protein in healthy silkworm, inoculated silkworm and antibacterial plant solution treated becomes more pronounced as the diseases progresses. High weight of protein was observed in the case of *Aloe barbedensis* (AKP 3) at the end of 8th day from the bacterial inoculation.

This would probably indicate that suppression of pathogen and boosting of immunity, the synthesis of proteins were greatly increased.

Table 4. Effect of aqueous extracts of *Aloe barbedensis* (AKP 3), *P. corylifolia* (AKP13) and *Bougainvillea spectabilis* (AKP 9) on the total protein content(mg/ml) in bacterial infected silkworm.

Treatment	*Days post inoculation*							
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
AKP 3	*16.17*	*20.26*	*23.75*	*28.55*	*31.95*	*36.75*	*36.03*	*35.27*
AKP 13	*16.28*	*20.21*	*23.61*	*27.05*	*29.56*	*34.27*	*34.14*	*34.05*
AKP 9	*16.33*	*20.05*	*23.58*	*26.88*	*29.12*	*34.69*	*34.19*	*33.96*
Inoculated control	*16.26*	*20.14*	*24.22*	*23.58*	*22.16*	*21.59*	*20.98*	*20.25*
Healthy control	*16.31*	*20.19*	*23.54*	*26.74*	*28.53*	*30.34*	*32.83*	*33.73*

Conclusion

Among the tested 13 antibacterial plants, *Aloe barbedensis* (AKP 3) has given encouraging results in containment of *bacillus* in silkworm. The *Aloe barbedensis* plant product can be recommended to the farmers for the prevention of crop loss due to the falacherie after conducting large scale field trials in the farmer's fields.

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