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Superiority of Azadirachtin Over Other Bio Chemical Active Compounds in Controlling Nuclear Polyhedrosis (Grasserie Disease) of Silkworm, *Bombyx Mori* Linnaeus

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Abstract

Purpose: The study was focused on to find out most cost-effective, user-friendly bio active chemicals among available one to control Grasserie.

Methodology: Simple rearing (both favourable and unfavourable rearing season) and dusting method.

Findings: Azadirachtin is more effective among other active biochemical counterparts.

Unique Contribution to Theory, Policy and Practice: As traditionally available disinfectants are of high cost and hazardous to its user as well to the environment, this type study is unique in its application and investigation. The Allicin and Curcumin were found to be similar in action in comparison to Azadirachtin particularly in unfavourable rearing season. However, Azadirachtin was found to be more effective in both favourable and unfavourable rearing season in comparison to its other counterparts.

Keywords: *Bombyx mori* L, Nuclearpolyherosis (Grasserie Disease), Bio-Active chemicals, Azadirachtin, Curcumin, Allicin

A. Background Information and Theoretical Framework:

Sericulture is one of the most ancient and important cottage industries of India, especially in the state of West Bengal where both mulberry and non-mulberry silkworms are reared in commercial scale. Presently more than 8.8 million farmers are engaged with silk industries in Indian subcontinent. West Bengal produced about 1632.638 M.T. of mulberry raw silk during 2021-22 (Anonymous, 2022). Therefore, it becomes essential to boost up them with recent developments and achievements in sericultural research for upliftment of the industry. The present study will focus on the comparative analysis of bio chemical control of Nuclearpolyherosis (Grasserie Disease) in Silkworm *Bombyx mori* Linnaeus in respect to the superiority of action of Azadirachtin to its other counterpart: Curcumin and Allicin. Both the aqueous and alcoholic extracts of Azadirachtin was found to be more effective than its other counterparts in unfavourable rearing seasons, which is the major finding of this study. The uniqueness of this type of bio chemical control of Grasserie disease which is not only a cost effective and eco-friendly technique, but also it is less hazardous and user friendly controlling procedure of Grasserie. Therefore this extensive study may help the stakeholders to step ahead on the progression of sustainable agriculture while avoiding crop loss by means of cost effective pathogen management technology.

Natural silk is produced in more than 60 countries all over the world. The main silk producing countries of the world today are China, India, and Japan, South and North Korea and a few states of Russia. Next in importance come Italy, Bulgaria, Brazil, Iran, Turkey and Thailand where sizeable quantities of raw silk are produced. Spain, Greece, Romania, Syria, Hungary and Taiwan come under the minor countries where sericulture is practiced. China and India contributed 1, 04,000 M.T. (81.89%) and 19,690 M.T. (15.5%) respectively of the global silk production in 2022 amongst 58 registered sericulture practicing countries in the world. Mulberry sericulture is traditionally practiced in West Bengal from the medieval period. In the 13th century at the time of Muslim regime Bengal silk came to prominence and even it was exported to European countries. The British East India Company organized the development of silk industry in West Bengal and up gradation of it. West Bengal produces both mulberry and non-mulberry silks and the mulberry silk production is highest in West Bengal. In West Bengal 4-5 commercial seasons are followed in a year. The climatic conditions of West Bengal may broadly be divided into the hot dry period (March-early June), the monsoon with high temperature and high humidity (late June–October) and the winter (November–February). The southwest monsoon has a great impact on the climate of the state. The intensity of the monsoon determines the prospects of sericulture to a considerable extent. In general, the areas located in the plains of West Bengal are very hot and humid during summer and fairly cold and dry during the winter. These conditions affect both growth of mulberry plant and rearing process of silkworm (Deb *et al.*, 2022).

Table: 1. West Bengal sericulture at a glance (2021-22).

Total Plantation area	38874 acre
Raw silk production	2332.78 M.T.
Silk waste	670.9 M.T.
Farmers Engaged	106490 No.
Villages	2500 No.
Cocoon production	25874.37 M.T.

Table: 2. District wise sericulture at a glance (2021-22) with special reference to Malda, Murshidabad and Birbhum districts of West Bengal.

	Malda	Murshidabad	Birbhum
Total plantation area	6960.34 acre	18955.66 acre	12958 acre
Raw silk production	576.65M.T.	978.54M.T.	777.59M.T.
Silk waste	183.51 M.T.	286.76 M.T.	200.63 M.T.
Employment	64832 No.	182496No.	123665No.

West Bengal, the major silk producing state in Eastern India occupies a unique geographical position (85.8°-89.8° East, 21.5°-27.3° North) and mulberry silkworm rearing is practiced mainly in the districts of Malda, Murshidabad and Birbhum. The climatic condition of West Bengal may broadly be divided into the hot dry period (from March - early June); the monsoon with high temperature and high humidity (from late June – October) and the winter (from November – February). The southwest monsoon has a great impact on the climate of the state. The intensity of the monsoon determines the prospects of sericulture to a considerable extent. In general, the areas located in the plains of West Bengal are very hot and humid during summer and fairly cold and dry during the winter. These conditions affect both growth of mulberry plant and rearing process of silkworm. In plains of West Bengal, rearing of seed and commercial crops is a round the year process. The commercial rearing seasons are February – March (*Chaitra*), March - April (*Baishakhi*), June - July (*Shravani*), August-September (*Aswina*), November - December (*Agrahayani*). Among the five commercial crops, November - December (*Agrahayani*),

February - March (*Chaitra*) seasons are considered as favorable for silkworm rearing when the temperature is optimum. March - April (*Baishakhi*) crop is also considered comparatively favorable season due to prevailing moderate temperature with low humidity during late March and early part of the April. The other crop seasons like, June - July (*Shravani*) and August - September (*Aswina*) are considered as unfavourable, when both temperature and humidity remain high, specially prevailing high humidity is the major constraint resulting in crop loss.

Nevertheless, the cocoon production is comparatively low due to various reasons of which disease is noteworthy. Silkworms suffer from various diseases like pebrine (microsporida), flacherie (bacteria), Grasserie (virus) and muscardine (fungus). These pathogens have a great role in deterioration of sericulture industry. Further, most of the commercially silkworm breeds exploited in India are prone to infection and silkworm breeds are reared in India since decades without any major change. As a result, they lose their tolerance against diseases (Deb *et al.*, 2021). Crop loss due to infection with various diseases was seen up to 20-40% (Keshan *et al.*, 2015). Among silkworm diseases, Grasserie, a viral disease of silkworm, *B. mori* L has been causing great economic loss estimating around 70-80% of the total loss in all silk producing countries. Thus, viral disease of silkworm is a great threat in the sericulture industry at present. The incidence of Grasserie was 18.6 % during *Shrabani* crop (June – July), 17.8% in *Aswina* crop (August – September), 8.5% in *Agrahayni* crop (October - November) and 15.6% in *Falguni* commercial crop (February) in Murshidabad district in 2020-21. Incidence of Grasserie ranged from 2.3-3.1% in Malda and an average 6.3% crop loss was reported due to Grasserie in Birbhum district (Anonymous, 2022).

This viral disease is presently called ‘Nuclear polyhedrosis’ caused by Nuclear Polyhedrosis Virus (NPV) (Family: Baculoviridae). However, the traditional chemical control method is insufficient to control grasserie and there comes a need to switch on to the unconventional techniques. Therefore biochemical mode of treatment using indigenous active compounds like Azadirachtin, Curcumin and Allicin is proposed, Besides, the detailed study in both favourable and unfavourable rearing seasons including predisposing factors for development and transmission of the disease at present scenario as well as screening of best bio active chemicals in this context will be studied with suggested as preventive and or controlling measures for application in the field to help the industry as well as the stakeholders.

B. Materials and Methodology:

High Temperature above 28 °C and low temperature below 20°C influence the high risk of occurrence of Grasserie disease. Fluctuation in temperature is also boost up the incidence of Grasserie.

Following materials have been taken into consideration for this study

1. The silkworm breed: Nistari (N) multivoltine

2. Rearing house and rearing appliances
3. Mulberry leaf (*Morus alba*), S1635 variety and
4. Bio Chemical active compounds (Azadirachtin, Curcumin and Allicin) for different experiments.

Silkworm Eggs

The egg of *B. mori* L multivoltine (Nistari) was taken from Silkworm Breeding and Genetics Laboratory of Central Sericultural Research and Training Institute, Berhampore, West Bengal.

Methodology applied during the study includes:

1. Rearing of different silkworm breeds and hybrid in different seasons of the year
2. Detailed survey of grasserie disease in three silk producing districts of West Bengal: Murshidabad, Malda and Birbhum
3. Histological, Electron microscopical and innate protein profile study
4. Isolation, purification and invasion of *BmNPV* in the healthy silkworm larvae to observe the changes morphometrically, anatomically and biochemically
5. Biochemical, ecofriendly and cost effective preventive measure of the disease

Rearing

The rearing was conducted in four commercial seasons namely

1. Season 1 (S1)- February – March
2. Season 2 (S2)- May - June
3. Season 3 (S3)- September – October and
4. Season 4 (S4)- November – December

Seasons 1 and 4 are called favourable seasons and remaining two seasons (S2 and S3) are called unfavourable seasons depending upon the environmental conditions for silkworm rearing. Rearings were conducted at Central Sericultural Research and Training Institute, Berhampore, West Bengal. The eggs of multivoltine Nistari (N) were collected from Silkworm Breeding and Genetics Research Laboratory of Central Sericultural Research and Training Institute, Berhampore, West Bengal. A standard model rearing house (24'× 15'×14') including fly room (8'× 15'×14') with 6 varandah was used for the experiments. Plastic Rearing tray with measurement of 3' × 2' ×2'', were used for rearing. Rearing rack used in the experiments was 11'× 2'× 8'. Other appliances used during rearing and the experiments were Chopping board made of wood, steel knife for chopping, Ant wel, Foam pad, Bed cleaning nets made of cotton, feather and foot sprayer. *Chandraki* or bamboo made mountage measuring about 6'×4' was used for mounting of silkworm (Saha *et al.* 2008). The larvae were fed with tender leaves of *Morus alba* (Mulberry). Particularly S1635 variety was used as it is a high yielding variety with a leaf yield of 30tonnes/ha/yr. This variety is also very much popular among the farmers of West Bengal. Tender leaves

were fed to the Ist and IInd instar larvae but the IIIrd one was fed with little medium leaves and the IVth and Vth ones were provided with mature leaves.

Rearing Schedule

Rearing was conducted in two phases. First in the favourable season, February–March and November–December and the second in the unfavourable season that is May–June and September–October. All the recommended package of practices of rearing was followed. The rearing was followed after Krishnaswamy (1978). At first the eggs were washed in 2% formalin solution for surface disinfection. Rearing house as well as rearing appliances was also disinfected by spraying with 5% bleaching solution. The eggs of Nistari were collected and kept for all the seasons for uniform hatching. When the eggs became pigmented during incubation, all the eggs were taken in a black box which was followed according to the standard black boxing method. After 48 hours the eggs were exposed to light for uniform hatching. Brushing of silkworm larvae is a process of separating the newly hatched larvae from the empty egg shells or egg sheets and transforming them to rearing bed. On the hatching day the egg sheets were exposed to light in the morning and at least one hour of exposure can result uniform hatching. Chopped tender mulberry leaves were provided (size: 0.5cm²) by sprinkling as thin layer over the hatched larvae. The silkworm larvae were kept for 10-20 minutes as such. The larvae were then transferred to rearing trays providing optimum spacing. Wet pad of foam and paper greased with paraffin was provided in the tray to maintain the optimum humidity. Larval stages of silkworm could be divided into five instars and during this period moulting occurs four times. The first three instars are called young age or *chawki* and the last two instars are called late age. The young age larvae were fed with tender leaves of mulberry plant and the fourth instar larvae were fed with mature leaves whereas the fifth instar larvae were given with cut twigs and mature leaves throughout the rearing and it was followed in all the rearing seasons.

Azadirachtin: Molecular formula: C₃₅H₄₄O₁₆, Molecular weight: 720.7

It is an active compound of Neem oil found in seeds of Neem tree (*Azadiracta indica*). Ranging from dark yellow to brown in colour, Azadirachtin is an active natural pesticide used for several years in Indian subcontinent. Azadirachtin is the most active component for repelling and killing pests and parasites from ancient time. It can be applied in wide range to crops and ornamental plants for controlling insects and pests. Azadirachtin is an amorphous dust soluble in water and alcohol or emulsifiable concentrates. Azadirachtin can repel and reduce the feeding of Nematodes. It has been observed that 90% of Azadirachtin dose is excreted out by the insects within seven hours, and remaining dose is enough to reach the target. In water, half-life of Azadirachtin ranges from 48 minutes to 4 days. It also breaks down rapidly in plant body and the half-life is 1-2.5 days. The remaining components are broken by microbes and environmental elements. Azadirachtin is nontoxic to most animal and plant except fish and other aquatic animals. It is commercially available and economically cost effective easy to use compound. It has been

purchased from T. Stanes and Company Ltd. Coimbatore, Tamil Nadu, India, for all the experiments.

Allicin: Molecular formula: $C_6H_{10}OS_2$, Molecular mass: 162.28g/mol

It is an active compound of Garlic (*Allium sativum*). It is an organo sulphur compound found in Alliaceae family plants. Anti-inflammatory property of Allicin has made it more accepted in bio medical research. Allicin quickly disintegrates into series of sulphur compounds which are able to control various activities. Allicin is an integral part of defence mechanism against attack by pests and pathogens in plant. Allicin is a defence molecule of Garlic with broad spectrum antimicrobial properties. Allicin can react with thiol groups and can inactivate microbial essential enzyme system. Besides bacteria, the effects of Allicin have been investigated in fungi and protozoa and even in viruses. Commercially available Allicin is water and alcohol soluble and easy to use for the stakeholders. It has been commercially purchased from Vihaan Foddertech, Siddhapur, Maharashtra, India, for the present experimental purposes.

Curcumin: Molecular formula: $C_{12}H_{20}O_6$, Molecular mass: 368.38g/mol

Curcumin is a yellow phytophenol pigment and active compound isolated from Turmeric (*Curcuma longa*), with a variety of pharmacological properties. It can block the formation of reactive Oxygen species resulting in inhibition of unnatural growth of cells. The desirable preventive or putative therapeutic properties of Curcumin have also been considered to be associated with its antioxidant and anti-inflammatory properties. Curcumin have antioxidant properties. It is therefore highly explored in cancer research arena. Laboratory research clearly indicates that it can slower down the progression and spread of cancerous growth. The major biochemical activity of Curcumin is mediated through its properties to inhibit cyclooxygenase -2 and lipoxygenase. It can induce Nitric Oxide synthesis and down regulation of protein kinase C. Improper upregulation of above stated components are associated with pathophysiology of certain animal cells that results in improper growth and development. Curcumin is a beta diketone compound found in the root stuff of turmeric plant. It is a well-tested hetero protective flavouring bio pigment having intraceutical and antifungal properties. Factors that enhance the activities of Curcumin are absence of potent and selective target specific activity, limited tissue distribution, low bio availability and extensive metabolism specificity. It has been purchased from Sangam Agro-Chemicals Pvt. Ltd. Gurugram, Haryana, India, or all the present experimental purposes. Curcumin in excess dose has less lethal effect as it is eliminated in faeces 90% or above.

Application of Disinfectants: Five sets of treatment, each with three replications along with control set was kept in the rearing room and larvae were brushed normally on the paraffin sheet and fresh mulberry chopped leaves were fed to the silkworms and allowed the silkworm for development. Hatching% were counted and recorded. Temperature and R.H% were also recorded. The IIIrd instar larvae were counted after undergoing their second moult and 100 larvae were kept for each treatment and replication. Then larvae

kept in five trays were dusted with different bed disinfectants whose compositions are given below:

Experimental Layout: This work has been conducted at Silkworm Breeding and Genetics Research Unit, Central Sericultural Research and Training Institute, Berhampore, West Bengal, India for five consecutive rearing seasons. The experiments were laid out in completely randomised design with below stated treatments replicated five times, with untreated control sets for each experiment.

Method of application: Before the commencement of silkworm rearing the appliances were sundried and rearing room along with rearing appliances were thoroughly cleaned and dried up using 2% Bleaching Powder solution and Absolute Alcohol. The entire room was later disinfected following standard protocol (Dandin *et al.*, 2003). The rearing room was kept air tight for 24 hours and after that the room was kept open and used for rearing. The temperature during the incubation of silkworm eggs was ranged from 23-25 °C and the relative humidity was ranged between 85-90%. Complete hatching took place after few days of incubation. New leaves and tender shoots of Mulberry were put over the hatching larvae, which crawled up the leaves and twigs, and then these were removed with silkworms to the rearing trays. Rearing schedule (Krishnaswamy, 1978) was continued till the end of second instar larvae enters moulting.

The third instar larvae were provided with chopped tender Mulberry (*Morus alba*) leaves (S1 variety) of required quantity and quality. The Mulberry leaves for feeding of Silkworm larvae were cut freshly every morning, and covered with wet clean and clear muslin cloth to protect that from loss of water. The leaves were cleaned and given to the first and second instar larvae as strips or buds. Afterwards, the whole leaves were distributed in a usual manner four times a day, till the beginning of fourth instar. Regular cleaning of rearing beds was carried out by removing uneaten leaves and faeces, to avoid infection spreading. After thirty minutes of initial feeding, 90 larvae were transferred to each experimental tray in each experimental tray with the mulberry leaves. Bed disinfectants were dusted once 30 minutes after resume from each moult and an additional dusting was done on 5th day of the fifth instar after bed cleaning. For dusting muslin cloth was used. During the course of rearing, disease wise larval mortality were recorded. Mortality was also recorded during harvesting of cocoons.

Observations and Data collection: In this study, data pertaining to Effective Rearing Rate (ERR) %, Single Cocoon weight, Single Shell weight and Shell (%) were recorded. Chemicals and botanicals are screened as inducer was dusted with selective dose to the larvae after resuming from 4th moult (1st day '0' hr). Dusting was done 3-4g/ sq. ft. of bed area on silkworm body. Dusting was done on silkworm larvae after each moult 30-45 minutes before the resumption of feed. One additional dusting was done on the 4th day of Vth instar after bed cleaning. On the appearance of disease symptom, dusting frequency increased every day. Observations like Larval mortality percentage (%) were recorded till the formation of cocoon. Mature larval weight (wt.), Cocoon wt., Shell wt., Effective

Rearing Rate (ERR) % was observed and recorded. The data was analysed statistically to verify the result.

C. Results and Observations:

Both the favourable and unfavourable rearing seasons are taken into consideration for the study. Both the alcoholic and aqueous solution of azadirachtin, allicin and curcumin (0.5 μ /ml) was used for each treatment sets in the laboratory set up. The most interesting and remarkable finding of this extensive study comes out from the observations of unfavourable rearing seasons. The total experimental setup showed a prominent response toward Azadirachtin in comparison to the other bioactive compounds. The initial larval weight was recorded 0.5852g and the final reading ranges from 3.9045g to 4.0466g (Table: 3 and 4) in favourable rearing seasons whereas in the unfavourable rearing season it was recorded in between 2.9143g to 3.046g (Table: 5 and 6). Considering both the sides it was found that Azadirachtin treatment showed significant increase in growth in comparison to other counterparts.

Similarly the cocoon measurements ranges between 1.8489g to 1.8489g in favourable rearing season (Table: 3 &4) and the same in the unfavourable rearing season was recorded in between 0.9881g to 1.0271g (Table: 5 & 6). The highest value was recorded in Azadirachtin treatment in comparison to the remaining counterparts in all the rearing seasons.

In comparison to the unfavourable rearing season, the favourable rearing season showing significant development in Azadirachtin treated experimental sets in all the parameters.

Table: 5 & 6, showing the data in respect to cocoon shell ratio in both rearing seasons which is significantly lower in Allicin (18.97% to 20.97%) and Curcumin (19.43% to 20.97%) treatment sets of silkworm larvae in comparison to the Azadirachtin (22.12% to 22.82%) treatment counterparts. The silk gland development in favourable rearing season after treatment with both aqueous and alcoholic extracts clearly discriminates the superiority of Azadirachtin (0.8600g to 0.9769g) over its two bio active chemical compounds: Allicin and Curcumin (Table: 3&4). Growth index in the unfavourable rearing seasons after the treatment with aqueous (4.3781) and alcoholic (4.414) extracts of bio active chemicals identifies the superiority of Azadirachtin over its other counterparts (Table: 5 & 6).

Table.3 Effect of alcoholic extracts (0.5µg/ml) of botanics on some growth characters of *B. mori*. L in favourable rearing seasons, in laboratory set up.

Growth Characters Treatment	Initial weight of the 5 th instar larvae (g)	Final average larval weight(g)	Range	Growth index	**	Average gland weight (g)	±SEM	*	Average larval weight (g)	±SEM	*	Gland weight ratio of larval weight %	*	Fresh cocoon weight (g)	S	Pupal weight (g)	S	Cocoon shell weight (g)	*	Cocoon shell ratio %	S
1 Azadirachtin	0.5852	3.9045	2.9192	4.9881	A	0.8600	0.011215	A B	2.9887	0.013189	A	25.43%	C D	1.8489	A	1.8988	A	0.4901	A	24.50%	A
2 Allicin	0.5852	3.2639	2.6302	4.4668	B	0.3594	0.015322	C	2.3652	0.018111	B	23.06%	D	1.4618	A	0.9612	A	0.2806	C	22.97%	A
3 Curcumin	0.5852	3.2371	2.6919	4.3233	C	0.3583	0.015715	B C	2.3903	0.0162091	A	22.01%	D	1.4632	A	1.0052	A	0.2881	D	22.43%	A
4 Trichodermin	0.5852	3.2996	2.6636	4.1878	BC D	0.3052	0.017161	A B	2.0063	0.015495	A B	24.68%	C D	1.4223	A	1.0365	A	0.2858	A B	21.61%	A
5 Control	0.5852	3.2162	2.6129	4.3312	BC D	0.3399	0.011258	A	2.3365	0.013864	B	23.95%	A	1.4612	A	0.9864	A	0.2807	B	22.15%	A
LSD value at	0.11	0.14	0.01	0.12	B	0.09	0.04	A	0.11	0.14	B	0.11	A	0.14	A	0.10	A	0.04	A	0.02	A
0.05 alpha level	0.14	0.12	0.12	0.2690	C	0.1258	0.11	C	0.3774	0.11	B	4.662	C	0.1412	C	0.1314	A	0.0082	C	1.983	C
0.01 alpha level	0.01	0.01	0.032	0.3672	AB	0.014	0.01	C	0.312	0.14	A B	0.01	C	0.17	A C	0.011	A C	0.12	C	0.11	A C

Table.4 Effect of aqueous extracts (0.5µg/ml) of botanics on some growth characters of *B. mori*. L in favourable rearing seasons, in laboratory set up

Growth Characters	Initial weight of the 5 th instar larvae (g)	Final average larval weight(g)	Range	Growth index	*	Average gland weight (g)	±SEM	NS	Average larval weight (g)	±SEM	*	Gland weight ratio of larval weight %	*	Fresh cocoon weight (g)	NS	Pupal weight (g)	NS	Cocoon shell weight (g)	*	Cocoon shell ratio %	NS
Treatment																					
1 Azadirachtin	0.5852	4.0466	2.9716	4.7441	AB	0.9769	0.026558	A	3.0552	0.049737	A	39.43%	B	1.5271	A	1.8382	A	0.3590	A	24.77%	AB
2 Allicin	0.5852	3.2484	2.6084	4.1521	AB	0.6108	0.036701	A	2.3450	0.051914	A	36.63%	AB	1.3775	A	1.0892	A	0.2883	A	22.57%	A
3 Curcumin	0.5852	3.2584	2.6068	4.1974	C	0.6360	0.035211	A	2.3190	0.058585	A	35.07%	B	1.3082	A	1.0255	A	0.2827	AB	22.61%	AB
4 Trichodermin	0.5852	3.0893	2.4701	4.1890	BC	0.6387	0.036284	A	2.4725	0.052920	B	35.83%	B	1.2824	A	1.0206	A	0.2618	C	21.42%	B
5 Control	0.5852	3.2367	2.6062	4.1341	AB	0.5847	0.039229	A	2.3750	0.058915	B	33.04%	A	1.2622	A	0.9844	A	0.2778	B	22.01%	AB
LSD value at	0.11	0.14	0.01	0.12	A	0.09	0.04	B	0.11	0.14	A	0.11	B	0.14	A	0.10	B	0.04	B	0.02	A
0.05 alpha level	0.14	0.13	0.11	0.2855	C	0.1786	0.11	C	0.3949	0.18	C	0.7120	B	0.1275	A	0.1248	A	0.0082	B	2.0000	A
0.01 alpha level	0.01	0.14	0.09	0.4155	C	0.10	0.04	AB	0.10	0.11	AC	0.09	AB	0.11	AB	0.08	AC	0.01	A	0.14	AC

Table.5 Effect of alcoholic extracts (0.5µg/ml) of botanics on some growth characters of *B. mori*. L in unfavourable rearing seasons, in laboratory set up

Growth Characters Treatment	Initial weight of the 5 th instar larvae (g)	Final average larval weight(g)	Range	Growth index	**	Average gland weight (g)	±SEM	*	Average larval weight (g)	±SEM	*	Gland weight ratio of larval weight %	*	Fresh cocoon weight (g)	*	Pupal weight (g)	*	Cocoon shell weight (g)	*	Cocoon shell ratio %	N S
1 Azadirachtin	0.5852	2.9143	2.6291	4.3781	C	0.3130	0.01875	C D	2.9987	0.014489	BC D	23.51 %	C	0.9881	B	1.0088	B	0.3701	B C D	22.12%	A B
2- Allicin	0.5852	2.4692	2.0230	3.8988	AB	0.2494	0.01452	B C	2.2452	0.010121	AB	19.06 %	B C	0.6175	A	0.8512	A	0.1766	A B	18.97%	A B
3 Curcumin	0.5852	2.6654	2.2489	3.8543	BC	0.2583	0.01871	D	2.3803	0.010691	D	20.11 %	B C	0.3112	B	0.8052	B	0.1781	C D	19.43%	A B
4 Trichodermin	0.5852	2.2874	2.1436	3.7658	AB C	0.2032	0.01987	B C	2.1064	0.011765	AB C	19.78 %	B C	0.5824	B	0.8365	B	0.1958	A B	19.61%	A
5 Control	0.5852	2.7512	2.5154	4.1232	AB	0.2989	0.01655	A B	2.8265	0.012764	CD	21.92 %	A	0.7622	B	0.9764	B	0.2107	D	21.11%	A B
LSD value at	0.11	0.13	0.02	0.12	A	0.07	0.04	A	0.13	0.14	B	0.13	B	0.12	A	0.20	B	0.03	B	0.03	A
0.05 alpha level	0.14	0.13	0.11	0.2630	C	0.1158	0.12	C	0.274	0.12	C	3.662	C	0.1375	A	0.1914	B	0.0076	C	1.7 83	A
0.01 alpha level	0.01	0.01	0.032	0.3982	AC	0.013	0.03	C	0.212	0.13	AB	0.03	A B	0.11	A C	0.011	AB	0.13	A B	0.11	A C

Table.6 Effect of aqueous extracts (0.5µg/ml) of botanics on some growth characters of *B. mori*. L in unfavourable rearing seasons, in laboratory set up

Growth Characters	Initial weight of the 5 th instar larvae (g)	Final average larval weight(g)	Range	Growth index	*	Average gland weight (g)	±SEM	*	Average larval weight (g)	±SEM	*	Gland weight ratio of larval weight %	NS	Fresh cocoon weight (g)	*	Pupal weight	NS	Cocoon shell weight (g)	NS	Cocoon shell ratio %	NS
Treatment																					
1 Azadirachtin	0.5852	3.046	2.6716	4.414	B	0.9769	0.035558	BC	2.9552	0.040737	B	29.43%	A	1.0271	AB	1.0842	A	0.3990	AB	22.82%	A
2 Allicin	0.5852	2.244	2.5084	3.952	A	0.3108	0.030701	BC	2.4450	0.030914	B	20.63%	A	0.9775	A	0.8592	A	0.1881	AB	20.97%	A
3 Curcumin	0.5852	2.254	2.3068	3.397	B	0.4460	0.024211	C	2.5190	0.039585	C	25.07%	A	0.9082	B	0.8255	A	0.1908	AB	21.43%	A
4 Trichodermin	0.5852	2.889	2.2701	3.889	A	0.3875	0.021284	A	2.0725	0.021920	A	25.83%	A	0.8824	A	0.8206	A	0.1989	A	20.61%	A
5-Control	0.5852	2.867	2.5362	3.931	A	0.3987	0.028229	AB	2.9750	0.037915	B	24.04%	A	0.9622	B	0.9784	A	0.2378	B	21.70%	A
LSD value at	0.11	0.15	0.01	0.11	A	0.06	0.04	A	0.10	0.14	B	0.11	B	0.13	A	0.10	A	0.03	A	0.02	B
0.05 alpha level	0.14	0.14	0.11	0.1865		0.1586	0.11	C	0.314	0.17	B	0.6120	C	0.1175	C	0.1148	C	0.0082	A	1.713	C
0.01 alpha level	0.01	0.14	0.08	0.3155		0.10	0.04	C	0.101	0.11	AB	0.11	AB	0.10	AC	0.10	AC	0.11	AC	0.11	AB

Discussions and Conclusions:

The history of medicinal plants can date back to the origin of human civilization on earth (Torquato *et al.*, 2006). In the early times, natural products were directly used for the treatment of diseases, including viral infection diseases. Natural products have made great contributions to human health, many active constituents from natural products have been determined and their mechanisms of action have been elucidated. Natural plant products have been used as the foundation of several medical treatments in humans. Although modern aspects of Western medicine have become the forefront of clinical practice today, natural plant products continue to be used as remedies in alternative medicine throughout the world. It is estimated that 80% of individuals in developing countries depend primarily on natural products to meet their healthcare needs (Khurad *et al.*, 2012). Even in the United States it has been found that approximately one in three Americans uses natural medicinal products daily. It has been estimated that of the 877 small-molecule drugs introduced worldwide between 1981 and 2002, approximately 61% can be traced back to their origins in natural products. Natural products are not effective, but are relatively non-toxic and have therapeutic doses well below their toxic levels (Jiao *et al.*, 2012). Curcumin is one such molecule that has shown promise since time immemorial. Nonetheless, there exists a significant barrier towards the utilization of these natural plant products in modern healthcare due to stigmatization of these “natural” remedies. Although the mechanisms of natural plant products and other plant-based drugs may not be well understood, it is important to uncover their mechanisms of action and determining their effectiveness. This will lead to a much more wide- spread acceptance of these alternative forms of treatment and allow it to be used in mainstream medicine (Wang, 2015). Unlike the disease of higher animals, if silkworms get infected once, it is not possible to recover by any kind of treatment. Hence, the best way is to find out ways and means to prevent the occurrence of the disease. Though perfect preventive measures have not yet been developed obviously, one of the earliest methods of reducing the extent of disease is removal of disease affected worms during rearing (Dutta and Ashwath, 2000). Further most effective method of reducing pathogen load in silkworm rearing is the dusting of chemical beds disinfectants, viz. Labex, Reshme, Vijetha etc. In addition profuse using of lime during silkworm rearing also significantly improves the crop performances. A spray of 0.3% slaked lime solution in addition to usual disinfection procedure is recommended prior to rearing whenever high incidence of Grasserie was experienced in the preceding crop (Matsubara, 2001). The extensive applications of chemical pesticides could contribute to climate change, toxic residues, pesticide resistance, and a decline in the numbers and habitats of potentially beneficial natural enemies of insect pests (Kotikal *et al.*, 2015). Given these potential adverse effects, increasing efforts have been made to explore the possibilities of developing biocides for crop protection that are based on naturally occurring substances. Plant-derived insecticides, also called botanical insecticides, are widely accepted. Although pest mortality is considered as the most significant evaluation index for pesticides, botanical extracts may cause a variety of sub-lethal effects, such as the impairment of development, reproduction, and behavior, which overall may be very important (Chakrabarty and Manna, 2008). Neem-based insecticides containing azadirachtin have played an important role in crop protection. Currently, azadirachtin, a very complex tetranortriterpenoid, has proved to be the most promising plant ingredient for

integrated pest management due to its broad-spectrum pest control activity (De-Loof *et al.*, 2017). In addressing the mode of action of azadirachtin, we found that azadirachtin inhibited proliferation and reduced protein synthesis in Sf9 cells (derived from the ovary of *Spodoptera frugiperda*). Moreover, during the process of azadirachtin-induced apoptosis, cathepsin acts as a pro-apoptosis element in the lysosomal pathway. Furthermore, the ability of azadirachtin to induce apoptosis in insect cell line has been confirmed by a series of thorough studies (Baig *et al.*, 2018). As for its effects *in vivo*, azadirachtin acts as a strong insect growth inhibitor, and has been shown to interfere with the neuroendocrine and alimentary systems in *Lutzomyia longipalpis* (Benchamin. and Nagaraj, 2014). Boulahbel and Ibarra, (2015) reported, in *Drosophila melanogaster*, that azadirachtin could interfere with the central nervous system via inhibition of the excitatory cholinergic transmission and partial blocking of the calcium channel in the suboesophageal ganglion region, eventually resulting in an ant fraudulent effect. Moreover, it could also inhibit egg-laying behavior and affect walking in larvae of *Tuta absoluta* (Boulahbel and Ibarra, 2015). However, some of the powerful biological properties of azadirachtin, such as acting as a feeding deterrent and as an insect growth inhibitor, may pose a potential risk to beneficial arthropods, especially the honeybee and silkworm. Studies revealed that 3.2 mg/L azadirachtin could significantly inhibit the egg-laying behavior of bees. Given that such sub-lethal effects are important for development and survival, more information about the potential adverse effects of azadirachtin on economically important beneficial insect is clearly required (Krarup *et al.*, 2015).

In this study, clear development inhibitory effects of azadirachtin against 5th-instar larvae of silkworm were observed, and such inhibitory effects may be caused by apoptosis in the prothoracic gland (Romanelli *et al.*, 2016). However, pure azadirachtin could not induce apoptosis in the prothoracic gland *in vitro*, in contrast to the effects of 20-hydroxyecdysone *in vitro*, which suggests that 20-hydroxyecdysone may not be the direct target of azadirachtin in perturbing silkworm larval development. Subsequently, we found that azadirachtin could trigger a significant increase in intracellular Ca²⁺ release in the Sf 9 cell line (De-Loof *et al.*, 2017), which suggested that azadirachtin may exert its action through Ca²⁺ which affects the process of apoptosis in the prothoracic gland and growth regulation in silkworms (Wang, 2015). The present study provides information on mechanism of action of azadirachtin in perturbing growth regulation, which may help us to better understand the potential risks of azadirachtin in relation to silkworms. Growth rate and developmental period must be regulated together in concert to ensure that organs develop to the correct size and proportions (Krarup *et al.*, 2015). Once insect larvae reach a critical weight, the titer of juvenile hormones declines and is accompanied by the release of prothoracicotropic hormone, which causes the prothoracic gland to synthesize the molting hormone, ecdysone (Inoue and Osatake, 2017). After ecdysone is converted into its active form, 20-hydroxyecdysone, it terminate larval development and initiate metamorphosis (Baig *et al.*, 2018). Botanical insecticides, such as azadirachtin, are biodegradable and they also serve as lead compounds and pharmacological probes to help us better understand biochemical and physiological mechanisms (Ponnuvel *et al.*, 2017). Azadirachtin-mediated intracellular Ca²⁺ release may be involved in apoptosis of the prothoracic gland, and thereby leading to growth

disruption. The results of this study provide us with insights into the mechanism of inhibitory action of azadirachtin against nucleopolyhedrosis in silkworm.

Natural medicine is a valuable field of research to explore, extract and establish curative properties. However, a very little percentage of phytochemicals has been systematically investigated for their therapeutic potential. Natural products provide an unusual approach for the discovery of antiviral agents with remarkable pharmacological effects. At present, approximately 25% of the drugs prescribed are of plants origin. Many anticancer and anti-infective drugs are derived from plant products (Sharan *et al.*, 2016). Herbal practitioners use traditional plants since ancient times to heal several human and animal diseases especially in Asia. People still rely on traditional plants and their products for their health, living and primary health care in many parts of the world. Approximately 2500 medicinal plant species have been recorded globally to treat a myriad of afflictions and diseases. Polyphenols, alkaloids, flavonoids, saponins, quinones, terpenes, proanthocyanidins, lignins, tannins, polysaccharides, steroids, thiosulfonates and coumarins are prominent bioactive phytochemicals, which have been observed to combat viral infections (Keshan *et al.*, 2015).

The control and prevention of various infections during silkworm rearing helps to increase the silk productivity by preventing the mortality to a great extent. In a view, use of these active compounds of different botanics during rearing improves the larval survival by preventing the infections and also improves the productivity of the silk resulting in the sustainable developmental improvement of sericulture industry in Indian subcontinent.

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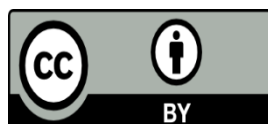
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