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Effect Of BmNPV Infection and Subsequent Oral Treatment Of Ethanolic Plant Extracts On Cocoon and Post Cocoon Characters In PM and CSR2 *B. Mori* L.

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ABSTRACT

In the present investigation BmNPV infection and subsequent oral treatment of ethanolic plant extracts effect was studied. After the inoculation and oral treatment of plant extractives observed the cocoon and post cocoon characters in both PM and CSR2 race. BmNPV inoculated group showed decreased the cocoon weight, shell weight, shell ratio, floss weight, filament length, filament weight and denier in both races. The maximum decrease was observed in CSR2 as compared to PM race. The significantly increased denier was observed in PM race treated with B. spectabilis as compared to control group i.e. 3.9 ± 0.09 and 2.9 ± 0.6 respectively. In other treated group C. longa and A. mexicana shows increase in all cocoon and post cocoon characters but not significant increase was observed as compared to their respective control group.

Key Words: BmNPV, *C. longa*, *A. mexicana*, *B. spectabilis*

INTRODUCTION

Sericulture is economically very important. But now a day various diseases are occur. Among the silkworm diseases, nuclear polyhedrosis virus (NPV) of Bombyx mori (BmNPV) is known to occur in all larval instars and more commonly in 4th and 5th instars during all seasons and causing 20-50% cocoon crop losses (Samson et al., 1990; Sivaprakasam and Rabindra, 1995).

In viral diseases two common diseases are found these are nuclear polyhedrosis and cytoplasmic polyhedrosis. Borellina virus cause nuclear polyhedrosis, which is principally the symptoms are skin become very thin, fragile and shiny and larvae become restless. Their skin rupture easily and a milky white fluid (haemolymph) oozes out though the holes.

NPVs remain in a stable infectious state in the environment even after death of NPV infected host and a large number of progeny NPV particles are released due to rupture of its cuticle, which transfers to other susceptible individuals (Richards et al., 1999). The released NPV particles must remain viable to occur secondary transmission, which is accomplished in part by the polyhedrin protein matrix that surrounds the infective units, the virions and provides some degree of protection against environmental degradation (Rohrmann, 1986).

Medicinal plants have become the focus on study in terms of validation of their traditional uses through the determination of their actual pharmacological effects. The control of infectious diseases is a seriously threatened by the continuous increases in the number of microorganisms that are resistance to the chemical antimicrobial drugs (Nenaah and Ahmed, 2011). The chemical based disinfectants and drug formations used for prevention and control of this disease are not economic, eco-friendly and have many limitations to be effective in open and outdoor rearing. Due to this reason disinfectants/drug formation are ineffective to control this disease at field level. Kagawa (1980), Reddy et al.,

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(1990), used the chemical disinfectants and antibiotics for managing the disease in silkworm. In view of high cost of chemicals and antibiotics and their hazardous consequences, plant extracts has been on the top priority for control of disease (Jespers and Waard, 1993, Kumar et al., 1999).

Now days the efforts were made to promote the use of botanicals as possible alternatives to treat infectious diseases (Jazani et al., 2009; Chanda, 2011). The natural products were found to possess promising antimicrobial activities when applied alone or in combination with conventional antimicrobial drug (Jazani, 2009). Kumar et al., (2009) and Manimeghalai et al., (2000) used plant products and succeeded to grasserie disease (caused by nuclear polyhedrosis virus) in mulberry silkworm, Bombyx mori.

The present work has been undertaken on the management of the viral disease causes damage silkworm. The ethanolic plant extracts used for management of disease the plants having antimicrobial activity showed the positive results**.**

MATERIAL AND METHOD

Isolation, Identification and purification of virus:

During rearing of silkworm in summer seasons, found the natural grasserie disease appeared, caused by nuclear polyhedrosis virus (NPV). The symptoms of this disease observed in the late age (Fourth and fifth instar). The NPV infected larvae showed the typical signs of infection such as inactiveness, intersegmental swellings, translucent appearance, bursting of body wall and oozing white turbid haemolymph.

Identification:

The larvae were isolated from the culture which are showed the symptoms of NPV infection and checked for virus infection by preparing the smear of haemolymph and mid gut tissue. The prepared smear also checked under the microscope to confirm the NPV infection and smear was stained with modified Azan stain Hubschman, (1962) and after fixation observed polyhedral inclusion bodies (PIBs) of NPV.

Isolation and purification of NPV:

The NPV infected fifth instar larvae were collected from the rearing trays and were triturated individually and crushed in mortar and pestle. The homogenate was filtered through muslin cloth. For the conformation of PIBs in homogenate, the filtrate was observed under microscope by preparing the smear on slide. For removing other microbes from the homogenate, it was purified by repeated centrifugation until clear white layer of PIBs was obtained. These obtained PIBs were stored in distilled water in refrigerator until their use.

Antimicrobial plants:

After screening against the BmNPV infection the three plants were selected which showed the positive results i.e. *C. longa, A. mexicana* and *B. spectabilis.*

Preparation of plant extracts:

Plants were collected from fields of Kolhapur District, Maharashtra, India. The taxonomic identification of the plants was made with available literature (Yadav and Sardesai 2002).

Plant extracts were prepared by the method of Alade and Irobi (1993) with minor modifications suggested by Ahmad and Beg (2001). The collected plant material washed with distilled water and shade dried at room temperature. The materials were grinded to fine powder with the help of mixer grinder. Then these powdered materials were used for preparation of ethanolic extracts by using 50g powder mashed in 100 ml absolute ethanol for 72 hours. The mixture was stirred every 24 hour using a sterile glass rod. At the end of extraction, each extract was concentrated in vaccuo at 30^0C and stored at 4^0C until further use.

Economic characters:

Cocoon weight:

The 10 cocoon were randomly selected for cocoon weight. The mean weight of cocoon was calculated and expressed in milligrams per shell.

Shell weight:

Randomly selected 10 cocoons were cut open with the help of sharp blade and the shell weight was recorded accurately and expressed in milligrams per shell.

Shell ratio:

Shell ratio denotes the total amount of silk available in a single cocoon and is expressed in percentage. It is the ratio between shell weight and cocoon weight, which were randomly selected. It is calculated by using following formula,

$$\text{Shell ratio} = \frac{\text{Weight of Shell}}{\text{Weight of cocoon}} \text{ X } 100$$

Floss weight:

It is the weight of floss recorded randomly selected 10 cocoons and the value was expressed in milligram.

Filament length:

It is the total length of the relable silk from a single cocoon. 10 randomly selected cocoons were cooked and reeled and the mean value of filament length in meters was calculated.

Filament weight:

It is the weight of silk reeled from 10 randomly selected cocoons and used for the assessment of filament length and it is expressed in milligrams.

Denier:

Denier represents the size of the silk filament, i.e., the weight in grams of 9000 meters of yarn per filament. It was calculated by using the formula,

$$\text{Denier} = \frac{\text{Weight of filament in grams}}{\text{Weight of the filament in meter}} \text{ X9000}$$

RESULTS

Effect of BmNPV infection and subsequent oral treatment of ethanolic plant extracts on cocoon and post cocoon character in PM and CSR2 *B. mori* L.:

The results on the effect of BmNPV inoculation and subsequent orally treatment of ethanolic plant extractives on cocoon and post cocoon characters in PM and CSR2 *B. mori* showed in **Table.**

1. Cocoon weight:

In control groups of both the race the PM showed 1034.2mg cocoon weight while in CSR2 cocoon weight was 1029.8mg. The inoculated of BmNPV caused the reduction in cocoon weight in both PM and CSR2. After the inoculation of *B. bassiana* the larvae fed on the ethanolic plant extract showed the decreased in their cocoon weight. In *C. longa* treated group the cocoon weight was reduced by 33.9% in PM and 25.4% in CSR2 race. In *A. mexicana* treatment the reduction in cocoon weight was observed in both PM and CSR2 race the reduction was 25.2% in PM and 12.5% in CSR2. The treatment of *B. bassiana* showed the reduction in cocoon weight by 9.33% in PM and by 6.8% in CSR2. The maximum reduction in cocoon weight was observed in *C. longa and A. mexicana* group of PM race and minimum reduction in cocoon

weight was observed in *C. longa and A. mexicana* group of PM race and minimum reduction in cocoon weight was observed in CSR2 treated with *B. spectabilis.*

The above results showed that the BmNPV infection caused the reduction in cocoon weight when compared to control groups in of both the race. Due to the treatment of ethanolic plant extract the cocoon weight was reduced than control groups but the reduction was less in plant treated groups when compared to BmNPV inoculated group. The cocoon weights get improved in plant extract treated groups which were nearer to the cocoon weight when compared to control groups

2. Shell weight:

In control group the CSR2 showed the more shell weight i.e. 164.9mg than the PM which was 124.5mg. The inoculation of PIBs responsible for reduction in shell weight in both PM and CSR2 races. The decrease in shell weight was observed in PM by 21.02% while in CSR2 the decrease shell weight was by 45.9%. The treatment of ethanolic plant extract after the inoculation BmNPV showed the increased shell weight in all group of PM race when compared to control group.

Table-1: Effect of BmNPV infection and subsequent oral treatment of ethanolic plant extracts on cocoon and post cocoon characters in PM and CSR2 *B. mori* L.

GROUP	RACE	COCOON WEIGHT (mg)	SHELL WEIGHT (mg)	SHELL RATIO (%)	FLOSS WEGHT (mg)	FILAMENT LENGTH (m)	FILAMENT WEIGHT (mg)	DENIER (d)
CONTROL	PM	1034.2 ± 87.5	124.5 ± 13.3	12.2 ± 1.4	26.8 ± 6.2	359.6 ± 76.9	84.6 ± 5.7	2.9 ± 0.6
	CSR2	1029.8 ± 192.6	164.9 ± 50.5	15.9 ± 3.5	15.5 ± 4.5	805.8 ± 109.4	145.2 ± 37.3	2.4 ± 1.1
INOCULATED	PM	556.2 ± 254.0 **(-44.16)** ***	98.3 ± 25.2 **(-21.02)** NS	8.0 ± 1.7 **(-34.6)** **	12.5 ± 5.7 **(-53.3)** ***	223.2 ± 112.6 **(-37.9)** *	65.2 ± 12.3 **(-22.96)** *	1.6 ± 0.7 **(-45.6)** ***
	CSR2	680.5 ± 76.31 **(-33.9)** **	89.2 ± 13.42 **(-45.9)** ***	10.69 ± 3.83 **(-32.72)** **	7 ± 3.5 **(-54.8)** ***	388.7 ± 116.25 **(-51.76)** ***	108.3 ± 49.75 **(-25.4)** **	0.96 ± 0.45 **(-60.33)** **
C. LONGA	PM	771.5 ± 180.8 **(-25.4)** *	154.7 ± 31.7 **(+24.2)** NS	11.7 ± 1.4 **(-3.9)** NS	11.3 ± 2.1 **(-57.7)** ***	350.5 ± 55.7 **(-2.5)** NS	85.7 ± 12.8 **(+1.26)** NS	2.3 ± 0.4 **(-18.94)** NS
	CSR2	995.2 ± 291.1 **(-3.3)** NS	147.5 ± 81.26 **(-10.5)** NS	14.32 ± 5.044 **(-9.86)** NS	7.6 ± 4.033 **(-50.9)** ***	643.9 ± 104.61 **(-20.0)** *	129.6 ± 50.66 **(-10.7)** NS	2.005 ± 0.76 **(-60.33)** **
A. MEXICANA	PM	773.6 ± 132.3 **(-25.2)** *	222.0 ± 55.7 **(+78.3)** ***	11.9 ± 2.0 **(-2.20)** NS	9.0 ± 2.4 **(-66.4)** ***	240.7 ± 50.2 **(-28.07)** *	75.0 ± 11.6 **(-11.34)** NS	2.3 ± 0.4 **(-18.94)** NS
	CSR2	900.8 ± 233.0 **(-12.5)** NS	131.2 ± 54.1 **(-20.4)** NS	14.3 ± 3.2 **(-9.9)** NS	9.1 ± 2.9 **(-41.2)** **	477.3 ± 79.7 **(-40.7)** ***	89.4 ± 25.0 **(-38.3)** *	1.7 ± 0.3 **(-30.57)** NS
B. SPECTABILIS	PM	931.3 ± 112.1 **(-9.93)** NS	189.6 ± 28.5 **(+52.26)** *	12.7 ± 1.4 **(+3.6)** NS	20.9 ± 8.6 **(+22.16)** NS	255.4 ± 46.5 **(-28.96)** NS	65.1 ± 8.4 **(-23.0)** *	3.9 ± 0.09 **(+36.14)** *
	CSR2	959.4 ± 234.94 **(-6.8)** NS	206 ± 49.98 **(+24.9)** **	17.79 ± 5.08 **(+11.9)** **	7.7 ± 4.55 **(-50.3)** ***	428.1 ± 192.89 **(-46.87)** ***	83.7 ± 44.53 **(-42.3)** *	1.78 ± 0.46 **(-26.44)** NS

Values with ± indicate mean with standard deviation, '+' and ' –' indicate percent increase and decrease *, **, *** and NS indicates the significance
level $P < 0.05$, $P < 0.01$, $P < 0.001$ and $P < 0.05$ respectively.

The decreased percentage was observed in CSR2 race plant treated groups when compared with control. In *C. longa* treatment the percent increase was observed in PM by 24.2% and percent decreased by 10.5% in CSR2. In *A. mexicana* treated group the increase in shell weight was observed in PM race by 78.3% and decrease in shell weight was observed in CSR2 by 20.4%. After the treatment of *B. spectabilis* the increase in shell weight was observed in both races in PM by 52.26% and in CSR2 by 24.9%.

The above results indicates that the PIBs causes infection to both races in PM and CSR2 in inoculated group. The maximum shell weight decreased was observed in CSR2 race than PM. But after the treatment of plant extract the percent decreased get reduced in *C. longa* treatment in PM race and *A. mexicana* treatment in CSR2 race than control groups but decrease in shell weight was less than the inoculated groups and other groups showed the improvement in their shell weights where were nearer to the shell weight of the control.

3. Shell ratio:

The higher shell ratio was observed in CSR2 i.e. 15.9% while the lesser was observed in PM i.e. 12.2%. In inoculated group the shell ratio was decreased because of the PIBs infection to the larvae of both races. The decreased in shell ratio was observed in PM by 34.6% while in CSR2 by 32.72%. But due to the treatment of ethanolic plant extract the decreased percentage in shell ratio was observed in *C. longa* and *A. mexicana* treated group in both races. The *C. longa* treated groups showed the reduced shell ratio by 3.9% in PM and by 9.86% in CSR2 race. After the treatment of *A. mexicana* plant extract to BmNPV inoculated larvae the decreased shell ratio was observed by 2.20% in PM and by 9.9% in CSR2. The increased in shell ratio was observed in *B. spectabilis* treated group by 3.6% and 11.9% in PM and CSR2 race respectively.

The above results revealed that the inoculation of PIBs caused the reduction in shell ratio which was higher in PM as compared to CSR2.

After the treatment of plant extract the maximum increase was observed in *B. spectabilis* in CSR2 race. In all the plant extract treated groups the decrease in shell ratio percentage when compared to control. But the shell was observed more when compared with the inoculated group.

4. Floss weight:

The more floss weight was observed in PM i.e. 26.8 mg while in CSR2 it was 15.5mg. The reduction in floss weigh was noticed in both races in PM race the floss weight was reduced by 53.3% and in CSR2 the reduction percentage was 54.7%. After the inoculation the larvae fed on the plant extract and which showed the reduction in their floss weight. In plant extract treated group the maximum reduction was noticed in *A. mexicana* treated in PM race while minimum was observed in *C. mulitiflorum* treatment.

The above results revealed that the after inoculation of BmNPV, PIBs the reduction in floss weight observed, but due to the application of plant extract the reduction of floss weight percentage was less as compared to inoculated group in both the races.

5. Filament length:

The higher filament length was observed in CSR2 as compared to the PM i.e 805.8m and 359.6m respectively. The inoculation of PIBs caused the reduction in filament length. The decrease in filament length was observed in PM by 37.9% while in CSR2 by 51.76% decrease was observed. The application of ethanolic plant extract caused the less reduction in filament length as compared to inoculated groups. The *C. longa* treatment showed the decreased of filament length in PM by 2.5% while in CSR2 by 20%. In *A. mexicana* treated group the reduction in filament length was by 28.7% in PM and by 40.7% in CSR2. The decrease in filament length in PM by 28.96% and in CSR2 by 46.87% was observed in *B. spectabilis* treated groups.

The above results showed that the inoculation of PIBs affect on the filament length in both the PM and CSR2 races. The reduction of filament length observed in inoculated groups but due to the subsequent application of plant extracts the reduction of filament length was less an compared to BmNPV inoculated groups.

6. Filament weight:

In the CSR2 race the high filament weight was more i.e. 145.2mg and lesser filament weight was observed 84.6 mg in PM race. The filament weight was decreased after the inoculation of PIBs in both the races of filament weight was observed. The decrease was observed in PM by 22.96% while in CSR2 by 25.4%. After the treatment of ethanolic plant extracts the *C. longa* treated groups showed the 1.26% increased weight in PM race while in CSR2 the weight was decreased by 10.7%. The reduction in filament weight by 10.7% and 11.34%was observed in PM and CSR2 races respectively. After the treatment of *C. multiflorum* the reduction in filament weight by 23.0% in PM and 42.3% in CSR2 was recorded.

From the above results it became clear that the PIBs inoculation causes the reduction in filament weight than control group in both races. But due to the application of plant extract the improvement in filament weight was observed.

7. Denier:

The more denier of fiber was observed in PM i.e. 2.9 while in CSR2 it was 2.4. After the inoculation the denier of decreased in PM race showed the 45.6% and CSR2 showed the 60.33% reduced denier values. The application of plant extracts showed reduction in denier than control but it was more than the inoculated groups. In *C. longa* treatment observed the decreased denier by 18.94% in PM and in CSR2 by 60.33%. After the

treatment of *A. mexicana* the denier was reduced by 18.94% in PM and 30.57% in CSR2 race. The increased denier was observed in PM race in *B. spectabilis* treated group which was by 36.14% while in CSR2 the decreased denier was by 26.44%.

The above result showed that the decreased denier was more after the inoculation of PIBs and the more decrease was observed in CSR2 than PM. In plant extract treated group decreased denier was less when compared to inoculated groups. The improvement in denier was observed in PM race in *B. spectabilis* treated groups. The plant extract treated groups showed the more or less similar denier when compared with denier of fiber of control group.

DISCUSSION

The observations of the present study as per the observations of Patil *et al.,* (1997). Who observed that the cocoon weight, shell weight, shell ratio, effective rate of rearing and fecundity get improved when mulberry leaves to *B. mori* larvae supplemented with *Parthenium* extractives in 1:20 ratio.

However, the increase was less as compared to control group. In PM race the maximum cocoon yield was observed in *A. mexicana* treated groups when compared with the inoculated group. However, the *A. mexicana* treated group showed the maximum cocoon yield in CSR2 race over the inoculated group. The treatment of plant extracts after the BmNPV inoculation in PM race the maximum cocoon yield was observed in *C. longa* treated group than the inoculated group. The *B. spectabilis* treatment yield maximum cocoons in CSR2 race as compared to the inoculated group cocoon yield. The use of above plants product reduced the mortality caused by BmNPV and improved the cocoon yield as compared to the inoculated group. The present results as per the findings of Manoharraj (1994) who reported the effectiveness of aqueous extracts of *P. corylifolia* against the BmNPV in *B. mori* L. The effectiveness of *Acacia suma* and *Caesalpinia coriaria* reducing the mortality due to the grasserie in third instar larva of *B. mori* (Manoharan, 1996). The reduction of grasserie disease was reported by using the *Curcuma longa* and chalk powder by Manimegalai *et al.,* (2000). The aggregation of polyhedral on treating with aqueous extracts of *T. terrestris* was reported by Sivaprakasam (1999). The aggregation may be due to the tannins and phenols present in plants. This is supported by Keating *et al.,* (1998), who reported that tannins and phenols may be able to bind directly with the virions and subsequently interfere with virus host cell interaction. The aqueous extracts of *A. suma* and *C. coriaria* showed the higher rate of aggregation and lesser mortality due to BmNPV. The higher aggregation of PIBs might be the reason for grasserie suppression when these were fed by worms reported by Manoharan (1996). The results obtained in the present study were similar with earlier reports on antifungal effect of *S. isoetifolium* revealed by Kumar *et al.,* (2009). The Kumari *et al.,* (2011) reported that the algal extract of *Turbinaria conides* reduced the mortality up to 75 to 85% due to the *B. bassiana* infection in *B. mori*. The economic characters enhanced and reduced the mortality due to BmNPV by using the extractives of *Psoalea corylifolia* and *Tribulus terrestrus* studied by Rajshekhargouda (1991) which supports the present finding. The present study showed that, the plant extract were effective in reduction in mortality up to 70-80% caused by the muscardine and BmNPV. Sivprakasan and Rabindra (1996) reported extrafoliation of *P. corylifolia* and *Tribulus terrestris* at 8000ppm to mulberry leaves ones during 3rd instar suppressed the grasserie disease by 80% which also supports the present study. Efficacy of seven plant products against the disease to silkworm larvae that were reported by Shivakumar *et al.,* (1995). The fruit of *A. marmelos* and leaves of *H. suveolese* and *E. odaratum* are the source of tannins, phenols and flavenoids which helps for the reduction in disease incidence due to bacteria and viruses reported by Bhaisare, (2007). The effects herbal extracts on the microbial pathogens causing flacherie and muscardiane disease in the mulberry silkworm *B. mori* L. reported by Isaiarasu *et al.,* (2011). The 2% aqueous extracts of *Aloe barbadensis, P. corylifolia* and *B. spectabilis* were found more effective in suppressing the virosis and reduced the mortality due to virus infection in *B. mori* (Kiran Kumar *et al.,* 2012).

After the treatment of ethanolic plant extracts in BmNPV inoculated larvae of PM and CSR2 the maximum shell ratio was observed in *B. spectabilis* treated group as compared to the other plant extracts in PM and CSR2 races. In all the plant extracts treated groups showed the increased shell ratio than the inoculated group. The Use of plant extracts not only reduces the mortality but also improves the larval weight, cocoon weight, shell weight, shell ratio, floss weight, filament length, filament weight and denier. Patil *et al.,* (1997) reported the extra foliation of leaf with extract of *Parthenium* increased the larval weight, ERR, cocoon weight, shell weight, shell ratio and fecundity in *B. mori.* The economic characteristics were increased due to the application of plant products with possible antimicrobial activity. This may also be due to the phagostimulant compounds present in these antimicrobial plants. The fruits *Aegle marmelos* and leaves of *Hyptis suaveolens* and *Eupatorium* contain the secondary metabolites in plants, which helps the reduction in mortality caused by BmNPV reported by Bhaisare (2007). The plants having antimicrobial activity used all over the world for reduction of pathogenic infection of various animals. Plants contain several classes of secondary metabolites like tannins, phenols, terpenes, caumarins, essential oils and flavonoids (Cutter, 2000; Mau *et al.,* 2001).

CONCLUSION

Hypertonic saline 3% was better than mannitol 20% as regard improvement of CT brain criteria of brain

edema, improvement of GCS and effect on renal functions. Also we can conclude that hypertonic saline 3% may replace mannitol 20% in the treatment of brain edema after traumatic brain injury due to its better efficacy and less adverse effects.

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Conflict of Interests

Authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1]. Ahmad, I. and Beg, A. Z. 2001. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. J. of Enth. 74: 113-123.

[2]. Alade, P. I. and Irobi, O. N. 1993. Antimicrobial activity of crud leaf extracts of *Acalypha wilkkensina.* J. Ethano. 39: 170-174.

[3].Bhaisare, S. S. 2007. The utilization of plant products in disease management of silkworm *Bombyx mori* L. Ph.D. Thesis, Shivaji University, Kolhapur, Maharashtra, India.

[4]. Chanda,S Kaneria,M and Nair,R. 2011.Antibacterial activity of *Psoralea corylifolia* L seed and aerial parts with various extraction methods.Res. J. Microbiol. 60: 124-131.

[5]. Cutter, C. N. 2000. Antimicrobial effects of herb extracts against *E. coli.* J.food protection.63: 601-607.

[6]. Hubschman, J. N. 1962. A modified Azan Staining Technique for Inclusion Body Viruses. Stain technol. 37: 379-380.

[7]. Isaiarasu, L., Sakthivef, N., Ravikumar, J. and Samuthiavelu, P. 2011. Effect of herbal extracts on the microbial pathogens causing flacherie and muscardine disease in the mulberry silkworm, *Bombyx mori* L. J. of Biopest. 4(2): 150-155.

[8]. Jazani, N. H., Zartoshi, M. BabaZadeh, H., Ali-daiee S. Zarrin and Hosseini, S. 2009. Antibacterial eefects of Iranian fennel essential oil on isolates of *Acinetobacter baumannii.* Pak. J. of Biol. Sci. 12: 738-741.

[9]. Jespers, A. B. K. and Waard, M. A. 1993. Natural products in plant protection. Eur. J. Plant Pathol. 99: 109-117.

[10].Kagawa, T. 1980. The efficacy of formalin as disinfectant of *Nosema bombysis* spores. J. Seric. Sci. Jpn. 49: 218-222.

[11].Keating, B., Polnarev, A., Steinberger, J., Timbie, P. 1998. J. Invet Pathol ApJ. 495- 580

[12].Kiran Kumar, K. P., Singh, G. P., Sinha, A. K., Madhusudhan, K. N. and Prasad, B. C. 2012. Antiviral action of certain medicinal palnts against AmCPV and their effect on cellular and biochemical changes in tasar silkworm, *Antheraea mylitta* D. J. of Medicinal. Plant. 6(1): 92-99.

[13].Kumar, R. S., Ramnathan, G., Subhakaran, M. and Inbaneson, S. J. 2009. Antimicrobial compounds from marine halophytes for silkworm disease treatment. Int. J. Med. Sci. 1: 184-191.

[14].Kumar, R. S., Ramnathan, G., Subhakaran, M. and Inbaneson, S. J. 2009. Antimicrobial compounds from marine halophytes for silkworm disease treatment. Int. J. Med. Sci. 1: 184-191.

[15].Kumar, V., Singh, G. H., Basui, A. M. Ahsam, M. M. and Datta, R. K. 1999. Germination, Penetration and inavasion of *Beauveria bassiana* causing white muscardine, The Italian J. of Zool. 66(1): 10-14.

[16].Kumari, S. S., Subba Rao, S. V., Mishra, S. and Murthy, U. S. 2011. Antifungal activity of *Turbinaria conoides* and Avaluation for the effective concentration against the infection of *Beauveria bassiana* in silkworm larvae. Res. J. of Micro.1-9.

[17].Manimegalai, S. A., Subramanian and N. Chandraram. 2000. Efficacy of bed disinfectantas botanical against grasserie disease of silkworm, *B. mori* L. Sericologia. 40: 585-590.

[18].Manoharan, S. 1996. Evaluation of certain botanicals for the management of grasserie disease of silkworm, *bombyx mori* L. M.Sc. (Sericulture) thesis, Tamil nadu Agriculture University, Coimbatore, India. 89.

[19].Manoharan, S. 1996. Evaluation of certain botanicals for the management of grasserie disease of silkworm, *bombyx mori* L. M.Sc. (Sericulture) thesis, Tamilnadu Agriculture University, Coimbatore, India. 89.

[20].Manoharraj, K. S. 1994. Effect of certain botanicals on the nuclear polyhedrosis virus disease of *Bpmbyx mori* L. M.Sc. (Sericulture) thesis, Tamilnadu Agriculture University, Coimbatore, India. 62.

[21].Mau, J. L., Chen, C. P. and Hsieh, B. C. 2001. Antimicrobial activity of extracts from Chinese Chive. J. Agric. Food. Chem.49: 183-188.

[22].Nenaah, E. G. and Ahmed, M. E. 2011. Antimicrobial activity of extracts and latex of *Calotropis procera* (Ait.) and synergistic effect with reference antimicrobials. Res. J. Med. Plant. 5: 706-716.

[23].Patil, R. R., Mahadevappa, M., Mahesha, H. M. and Patil, V. C. 1997. Phagostimulant effects of parthenium on mulberry silkworm (*B. mori* L.) *"First International Conference on Parthenium*

Management" Dharwad, Karnataka, India, Oct. 6-8., 81-85.
[24].Rajashekhargouda 1991. Studies on methods to increase silk yield of *B. mori* L. Ph.D. Thesis, Tamil. Nadu, Agricultural University, Coimbatore, India, 242.
[25].Reddy, S. V., Singh. B. D., Baig, M. Sengupta, K., Giridhar and Singhal, B. K. 1990. Efficacy of asiphore as a disinfectant against incidence of silkworm *Bombyx mori* L. Indian J. Seric. 29: 147-148.
[26]. Richards, A., Coxy, J., Speight, M. and Williams, T. 1999. Foraging in a pathogen reservoir can lead to local host population extinction. A case study of Lepidoptera- virus interaction. Oecologia (Berlin).118(1): 29-38.
[27].Roharmann, G. F. 1986. Polyhedrin structure. J. Gen. Virol.78: 1499-1513.
[28].Samson, M. V., Baig, M., Sharma, S. D., Balvenkatasubbaih, M., Sasidharan, T. O. and Jolly, M. S. 1990. Indian J. Seric. 29: 248-493.
[29].Shivakumar, G. R., Raman, K. V. A. , Reddy, K. V. R. , Magadum, S. B., Datta, R. K., Hussain, S. S. , Banerji, A. , Chowdhary, S. K. 1995. Effect of phytoecdysteroids on larval maturation and economic parameters of the silkworm. *Bombyx mori* L. Indian J Seric. 34:46–49.
[30].Sivaprakasam, N. 1999. Botanical formulation for management of grasserie disease of silkworm, *B. mori* L. Proceeding of *"National Seminar on Tropical Sericulture"* held during the 28-30th December.
[31].Sivaprakasam. N., Rabindra, R. J. 1996. Integrated disease management methods for grasserie in silkworm, *B. mori* L. Indian J. Seric. 35: 122-127.
[32].Sivaprakasan, N. and Rabindra, R. J. 1995. Incidence of grassirie in silkworm *Bombyx mori* (L) in selected districts of Tamil nadu. Indian J. Seric. 34(2): 100-104.
[33].Yadav, S. R., Sardesai, M. M. 2002. Flora of Kolhapur District, Shivaji Univerity Publication, pp. 32,39, 191 &193.

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