

UNIVERSITY GRANT COMMISSION

BAHADUR SHAH ZAFAR MARG

NEW DEHLI - 110 002

Annual / Final Report of the work done on the Major/Minor Research Project (Report to be submitted within six weeks after compilation of each work)

1. Project report No. 1st /2nd /3rd /final : Final
2. UGC Reference No. : F.No.33-367/2007 (SR)
3. Period of the project : From 01/04/2008 to 31/03/2011
4. Title of research project : "Utilization of herbals in the disease Management of silkworm *Bombyx Mori* L."
5. a) Name of the principal investigator : Dr. G. P. Bhawane
Department of Zoology
Shivaji University
Kolhapur – 416004 (MS)
- b) Dept. /University /college where the work has progressed : Department of Zoology,
Shivaji University,
Kolhapur-416004
6. Effective date of starting the project: 01/04/2008
7. Grant approved and expenditure incurred during the period of the report:
 - a) Total amount approved : Rs. 5, 06,200/-
 - b) Total expenditure in three years : Rs. 5, 06, 042/-
 - c) Remaining balance : Rs. 158/-
8. Report of the work done : (please see appendix A)

9. OBJECTIVES OF THE PROPOSAL:

1. Screening of different plants with antimicrobial activity against silkworm diseases.
2. Characterization of chemical compounds having antimicrobial activity from the plants.
3. Utilization of plant products having antimicrobial properties to be the permeable formulation of antimicrobial agent.
4. To study the impact of antimicrobial phytochemicals on the economic characters of silkworm *Bombyx mori*.
5. Studies on the effects of plant derived antimicrobial in biochemical, hematological, neuroendocrinological changes in silkworm *Bombyx mori*.
10. Has the progress been according to original plan of work and towards achieving the objectives if not, state reason: Yes
11. Please indicate the difficulties, if any, experienced in implementing the project:
No
12. If project has not been completed, please indicate approximate time by which it is likely to complete: Not applicable
13. If the project has been completed, please enclose the summary of the findings of the study: (please see appendix A).
14. Any other information which would help in the evaluation of the work done on the project. At the completion of project, the first report should indicate the output, such as

a) Manpower trained:

Project fellow appointed in this project has attended two workshops.

1. Workshop on "Techniques in plant and animal tissue culture" organized by KIT's College of Engineering Kolhapur.
2. National level Workshop in "Techniques in Biology" organized by Department of Zoology, Sri Pancham Khemraj Mahavidyalaya, Sawantwadi, Dist Sindhudurga.

b) Ph.D. awarded:

Project fellow appointed in this project has registered for her Ph. D. degree on the topic related with this project entitled "**Studies on antifungal and antiviral properties of plant extracts in disease management of *Bombyx mori* L**".

c) Publications of the results:

Papers published:

G. P. Bhawane, J. A. Chavan, S. M. Gaikwad, S. R. Aland, V. J. Kumbhar and N. T. Pawar (2010). Efficacy of ethanolic plant extractives on biochemical moieties and cocoon character of *beauveria bassiana* infected 5th instar larvae, *Bombyx mori* L. *Geobios*.37: 314-318.

Papers Accepted:

- i. G. P. Bhawane, J. A. Chavan, Y. S. Muniv, S. M. Gaikwad*, M. Goswami**. (2010). "Protein, Lipid and Carbohydrate profile in the mid gut of silkworm, *Bombyx mori* L. infected with *Beauveria bassiana* (Bals.) Vuill". *Indian Journal of Comparative Animal Physiology*.
- ii. Jyoti Ashok Chavan, Santosh Jalinder Patil and Ganesh Parashram Bhawane. (2010). Screening of aqueous plant extracts agaiection to Vth instar larvae of *Bombyx mori* L. *Journal of medicinal plant research*.
- iii. J. A. Chavan, S. J. Patil and G. P. Bhawane. (2011). Aqueous leaf extract of *Clerodendrum multiflorum* exhibit antifungal effect against white muscardine disease of silkworm, *Bombyx mori* L. *Bionano frontier*.

- iv. J. A. Chavan and G. P. Bhawane. (2011). Secondary metabolites of antimicrobial plants. *Bioinfolet*.

Papers communicated:

- i. Haemolymph protein profile in nuclear polyhedrosis virus inoculated and plant extractives treated silkworm *Bombyx mori* L.
- ii. Differential haemocyte counts of 5th instar silkworm *Bombyx mori* L. inoculated with NPV and treated with ethanolic plant extracts.

1. Paper presented in the conference :

- i. J. A. Chavan, Y. S. Muniv, S. M. Gaikwad, M. Goswami and G. P. Bhawane (2010). Protein, lipid and carbohydrate profile in the midgut of silkworm, *bombyx mori* L. Infected with *Beauveria bassiana* (bals.) Vuill in National Conference on “**Life Sciences with Special Reference to Environmental Biotechnology and Biodiversity**” organized by Vivekanand College, Kolhapur- 416 003 (2-3rd February 2010).
- ii. J. A. Chavan, S. M. Gaikwad*, S. R. Aland, V. J. Kumbhar, N. T. Pawar and G. P. Bhawane (2010). Efficacy of ethanolic plant extractives on biochemical moieties and cocoon character of *Beauveria bassiana* infected 5th instar larvae, *Bombyx mori* in State Level Conference on “**Recent Trends in Physico Chemical Sciences, Biotechnology and Biodiversity**” organized by Padmashree Dr. D. Y. Patil Arts, Commerce and Science College, Pimpri, Pune- 18 (12-13th February 2010).
- iii. J. A. Chavan, S. J. Patil and G. P. Bhawane (2010). Aqueous leaf extract of *Clerodendrum multiflorum* exhibit antifungal effect against white muscardine disease of silkworm *Bombyx mori* L. in “**XXXIII conference of Indian botanical society and international symposium on the new horizons of botany**” organized by Department of botany, Shivaji University, Kolhapur- 416 004 (10-12th November, 2010).

- iv. J. A. Chavan, S. J. Patil (2010). Screening of aqueous plant extracts against *Beauveria bassiana* infection to Vth instar larvae of *Bombyx mori* L. in National conference on "Global warming and conservation strategies" organised by Department of zoology, Lal Bahadur Shastri College, Satara-415 002 (21-22nd December, 2010).

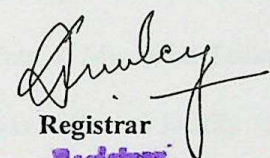
d) Other impact if any: NIL

Date 21-7-11


Principal Investigator

(Dr. G. P. Bhawane)

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Appendix – A

Report of the work done:

Methodology:

I. Selection of races of silkworm for the treatment of pathogenic organism:

- a. Multivoltine race Pure Mysore (PM)
- b. Biovoltine race CSR₂ (comparatively susceptible to infection)

Above races were obtained from Sericulture Grainage Center, Gadhinglaj, Dist. Kolhapur.

II. Selection of pathogenic organism for treatment:

Initially organisms from each group i. e. bacteria, fungi and virus is selected. Bacteria and fungi were made available from MTCC, Institute of Microbial Technology, Sector 39-A, Chandigarh – 160 036. India and BmNPV was collected locally from the grasseries infected larvae from silkworm rearers. In bacterial study three bacteria were selected.

Bacteria:

Bacillus thuringiensis: causes Sotro disease. Spore forming bacteria produce toxin at the time of sporulation. It is stored in parasporal diamond shaped bodies.

Staphylococcus aureus and *Pseudomonas aeruginosa*: These bacteria cause the Bacterial flacherie or Gastric injuri flacherie. Bacterial flacherie caused by the multiplication of bacterial in the alimentary canal which has become weaker due to bad environmental condition.

Fungi:

Beauveria bassiana cause white muscardine and the spores appear as white powdery speck.

Aspergillus flavipes cause brown muscardine and conidia are ovoidal in shape and are dirty brown in colour.

Virus:

NPV (BmNPV) *Bombyx mori* nuclear polyhedrosis virus multiplies and form polyhedra in the nucleus of various types of cells of the host (Malpighian tubules, silk gland, epidermis, fat bodies and mid gut). The BmNPV PIB's were used for infection.

III. Preparation of plant extract:

Plant extracts were prepared by the method of Alade and Irobi (1993) with minor modifications suggested by Ahemad and Beg (2001). Briefly 100 gm of each powdered plant material were soaked in 200 ml of 80% alcohol for 72 hours each mixture was stirred every 24 hours using sterile glass rod. At the end of extraction each extract was passed through Whatman filter paper 1. The alcoholic filtrate obtained was concentrated in Vacuo at 30°C and stored at 4°C until further use.

Besides this extraction is also made in diethyl ether as per method suggested by Muthuvelam and Raja (2008). Extractive obtained was stored at 4°C which was utilize in in the experiment.

IV. Determination of pathogenic organism selected for present study:

a. Preparation of bacterial suspension for treatment:

Working culture of *B. thuringiensis*, *S. aureous* and *P. aeruginosa* were made from transfer of a few loops of pure culture in to nutrient broth and incubated at 37°C for 24 hrs. The total bacterial count in the culture was evaluated using agar plate method. The standard count plate method involves serial dilution and plating technique. The series of dilution of bacteria were prepared with sterile saline as 10^1 , 10^2 10^3 -----
----- 10^9 (Jamuna, 2004).

b. Preparation of fungal spore suspension of *B. bassiana* and *A. flavipes* for treatment:

Pure culture of *B. bassiana* and *A. flavipes* was maintained as per standard procedure of Govindan (1998), Lakshmi (2003). A few loops of well sporulating culture from PDA plate were taken and suspended in sterile water. Serial dilutions were made from this by using sterile distilled water as 10^1 , 10^2 , 10^3 ----- 10^9 .

c. Preparation of viral PIB's suspension of BmNPV:

Isolation and purification of NPV:

The grasserie infected larvae of *Bombyx mori* were collected from the rearing trays. They were triturated with sterile distilled water with the help of glass homogenizer. The homogenate filtered with muscline cloth, filtrate observed under microscope by smear preparation on slide to confirm presence of PIB's. The filtrate was purified by repeated centrifugation until clear white layer of PIB's was obtained. With the help of Neubauers chamber number of PIB's/ml were determined from the stock suspension by making series of PIB's dilution in sterile distilled water as 10^1 , 10^2 , 10^3 ----- --- 10^9 (Mahulikar, 2003).

d. Determination of LC_{50} of each microbial suspension against PM and CSR2 race.

e. Treatment of pathogen to PM and CSR2 race of *Bombyx mori* used is as follows:

In case of bacteria, freshly collected cleaned leaves of mulberry (50 gms) deeped in 50 ml of different concentrations of bacterial suspension. Which are then dried at room temperature was provided to the respective groups of larvae in separate trays. For the treatment of fungus the 50 larvae of each group singly deeped in respective concentration of fungus spore suspension and for treatment of BmNPV leaves of mulberry (50gms) treated with 50 ml of BmNPV suspension which after shade drying given to the larvae of each group for infection. Larvae after treatment fed with the fresh

leaves of mulberry and the mortality due to microbial infection was counted up to 7 days of larval duration. Similarly 3 replicate for each treatment is prepared and LC_{50} value is determined for each microbes.

V. Testing of antimicrobial properties of plant extracts:

a. Mode of administration of plant extract:

PM and CSR2 race of *Bombyx mori* were used for testing antimicrobial activity of plant material. The twenty plants were selected for screening in the present study and ethanolic crude condensed products were used in 2000, 4000, 6000 and 8000 ppm concentration prepared in distilled water. The treatment of LC_{50} concentration was given to freshly moulted and two hours starved 5th instar larvae of both the races. After 6 hrs of treatment the larvae were segregated to appropriate groups for giving treatment of plant extractives. The plant extractives were given along with the mulberry leaves for which 50 gms leaves dipped in respective concentration of extractives and then shade dried before feeding to the larvae. Treatment is given for three days along with the morning food only.

b. Biochemical estimation of healthy infected and plant extract treated silkworm larvae:

1. The total protein content from healthy and infected silkworm larvae was determined by Lowry et al., (1951).
2. Total carbohydrate content from healthy and infected silkworm larvae was determined by using 3, 5 dinitrosalicylic acid reagent prepared as per the method of Noelting and Bernfield (1948).
- c. Total lipid content from healthy and infected silkworm larvae was determined by using Barnes and Blackstock (1973) method.

d. Haematological study of healthy infected and plant extract treated silkworm larvae.

Total haemocyte count (THC) and differential haemocyte count (DHC) was determined by Praful (1994).

e. Cocoon characters:

Cocoon weight, shell weight and shell ratio was calculated from healthy, infected and plant extract treated silkworm larvae.

f. Neuroendocrinology:

The whole mount of brain from normal control, inoculated control and plant extracts treated groups was prepared by using aldehyde fuchsin method stain (PAF) as per the methodology described by Dogra and Tondon (1964).

Results obtained:

1. LC_{50} value for each pathogen in both races:

- i. **LC_{50} value for bacteria:** In PM race LC_{50} value was observed 1×10^5 cells/ml and in CSR2 1×10^4 cells/ml after the inoculation with *B. thuringiensis*. In case of *S. aureus* infection the LC_{50} was observed at 1×10^5 cells/ml in PM and at 1×10^4 cells/ml in CSR2 race. In *P. aeruginosa* serial dilution of bacterial cells shows LC_{50} value 1×10^4 cells/ml and 1×10^4 cells/ml in the respective races.
- ii. **LC_{50} value for fungi:** In *B. bassiana* infection LC_{50} value observed in PM race was 1×10^6 spores/ml and in CSR2 was 1×10^5 spores/ml. In case of *A. flavipes* infection the LC_{50} observed were 1×10^5 and 1×10^4 spores/ml in PM and CSR2 respectively.
- iii. **LC_{50} value for Virus:** In BmNPV inoculation to silkworm at various dilution at 1×10^4 and 1×10^3 PIBs/ml shows the LC_{50} concentration in PM and CSR2 race respectively.

1. Screening of plants:

For present study, following plants were selected which are having antimicrobial activity. These are *Azadirachta indica*, *Curcuma longa*, *Aegle marmelos*, *Lantana camera*, *Mormordica charantia*, *Euphorbia pulcherima*, *Hyptis suaveolens*, *Clerodendrum multiflorum*, *Argemone Mexicana*, *Syzygium cumini*, *Terminalia arjuna*, *Annona squamosa* and *Bougainvillia spectabilis*. For screening 2000, 4000, 6000 and 8000ppm concentration of plant extract was used for both the races i.e. PM and CSR2 and results obtained were at 6000ppm concentration shows the reduction in mortality percentage. Therefore 6000ppm concentration was selected for further experiment.

For preparation of plant extracts two solvents were used, alcohol and diethyl ether. The alcoholic plant extracts showed the promising results but in case of diethyl ether plant extracts it does not showed the positive results. Therefore for further study only alcoholic plant extracts was selected and screened against antibacterial, antifungal and antiviral activity. The results obtained after screening are as follows:

Antibacterial activity:

Curcuma longa, *Lantana camera*, *Mormordica charantia*, *Euphorbia pulcherima*, *Argemone mexicana*, *Syzygium cumini*, *Terminalia arjuna*, *Hyptis suaveolens*, and *Bougainvillia spectabilis*.

Antifungal activity:

Azadirachta indica, *Curcuma longa*, *Clerodendrum multiflorum*, *Argemone mexicana*, *Syzygium cumini* and *Terminalia arjuna*.

Antiviral activity:

Azadirachta indica, *Curcuma longa*, *Mormordica charantia*, *Euphorbia pulcherima*, *Argemone mexicana*, *Syzygium cumini*, *Terminalia arjuna*, *Hyptis suaveolens*, and *Bougainvillia spectabilis*.

After the treatment of plant extracts decrease the mortality percentage and cocoon yield is high as compared to pathogen inoculated groups. The cocoon yield percentage after the treatment is as follows,

Cocoon yield in bacterial infection:

The silkworm inoculate with *B. thuringiensis* bacteria, treated with plant extracts shows the yield of cocoons in *Curcuma longa* (75%, 56%), *Lantana camera* (90%, 76%), *Mormordica charantia* (80%, 46%), *Euphorbia pulcherima* (75%, 53%), *Argemone mexicana* (70%, 53%), *Syzygium cumini* (90%, 63%), *Terminalia arjuna* (85%, 90%), *Hyptis suaveolens* (90%, 76%), and *Bougainvillia spectabilis* (85%, 63%) in PM and CSR2 race respectively.

The silkworm inoculate with *S. aureus* bacteria, treated with plant extracts shows the yield of cocoons in *Curcuma longa* (74%, 66%), *Lantana camera* (70%, 64%), *Mormordica charantia* (83%, 56%), *Euphorbia pulcherima* (85%, 73%), *Argemone mexicana* (77%, 63%), *Syzygium cumini* (89%, 73%), *Terminalia arjuna* (87%, 79%), *Hyptis suaveolens* (80%, 71%), and *Bougainvillia spectabilis* (85%, 67%) in PM and CSR2 race respectively.

The silkworm inoculate with *P. aeruginosa* bacteria, treated with plant extracts shows the yield of cocoons in *Curcuma longa* (77%, 60%), *Lantana camera* (74%, 61%), *Mormordica charantia* (73%, 66%), *Euphorbia pulcherima* (77%, 63%), *Argemone mexicana* (79%, 73%), *Syzygium cumini* (79%, 66%), *Terminalia arjuna* (67%, 66%), *Hyptis suaveolens* 79%, 61%), and *Bougainvillia spectabilis* (84%, 77%) in PM and CSR2 race respectively.

Cocoon yield in fungal infection:

After treatment of plant extracts the silkworms inoculate with fungal spores *B. bassiana* showed the increase the cocoon yield as compared to the inoculated control.

Azadirachta indica (70%, 65%), *Curcuma longa* (83%, 69%), *Clerodendrum multiflorum* (79%, 71%), *Argemone mexicana* (83%, 77%), *Syzygium cumini* (74%, 66%) and *Terminalia arjuna* (69%, 63%) in both the races PM and CSR2 respectively.

In *A. flavipes* inoculation after the treatment of plant extracts showed the decrease the mortality percentage and increases the cocoon yield as compared to the inoculated control. Yield of cocoon is more or less similar to the normal control groups. *Azadirachta indica* (80%, 75%), *Curcuma longa* (87%, 79%), *Clerodendrum multiflorum* (99%, 77%), *Argemone mexicana* (89%, 79%), *Syzygium cumini* (86%, 75%) and *Terminalia arjuna* (88%, 71%) in both the races PM and CSR2 respectively.

Cocoon yield in viral infection:

The rate of cocon yield was higher in plant extracts treated groups as compared to inoculated control in both the races. The percentage of cocoons in *Azadirachta indica* (70%, 61%), *Curcuma longa* (63%, 59%), *Mormordica charantia* (76%, 66%), *Euphorbia pulcherima* (66%, 62%), *Argemone Mexicana* (75%, 73%), *Syzygium cumini* (83%, 74%), *Terminalia arjuna* (61%, 61%), *Hyptis suaveolens* (85%, 80%), and *Bougainvillia spectabilis* (83%, 79%) in both the PM and CSR2 respectively.

From the above results it is clear that after the treatment of plant extracts there was reduction in the larval mortality and larvae spin like normal control and increase the percentage yield of cocoons in case of all the pathogens.

2. Characterization of chemical compounds:

Plants having antimicrobial activity and secondary metabolites like, Phenols, Tannins, Flavonoids, Alkaloids, Polyphenols etc. In the present study estimation of secondary metabolites like tannins and phenol content from the above selected plants were done. The results showed presence of tannin and phenol content from each plant

were submitted in second annual report. The other secondary metabolites present in selected plants are listed in table.

On this study one paper is accepted entitled:

- i) J. A. Chavan and G. P. Bhawane. (2011). Secondary metabolites of antimicrobial plants. *Bioinfolet*.

Table 1. Secondary metabolites in plants.

Sr. No	Botanical Name	Local name	Family	Plant part used	Phytoconstituents presents in plants
1.	<i>Azadirachta indica</i>	Neem	Meliaceae	Leaves	Azadirachtin, margosic acid, nimbin, nimbinin, nimbidin, tannins and flavonoids.
2.	<i>Curcuma longa</i>	Haldi	Zingiberaceae	Leaves	Curcumin, terpenoids.
3.	<i>Syzygium cumini</i>	Jamun	Myrtaceae	Seeds	Jambosine, tannins, phenols, and flavonoids.
4.	<i>Aegle marmelos</i>	Bael	Rutaceae	Fruits	Essential oil, terpenoid and alkaloid.
5.	<i>Bougainvillia spectabilis</i>	Kagadphul	Nyctaginaceae	Leaves	Quinonnes, saponins, triterpenoids, flavonoids, alkaloids, sterols, tannins and phenols.
6.	<i>Hyptis suaveolens</i>	Vilayati tulas	Labiaceae	Leaves	Flavonoids and triterpenoids.
7.	<i>Euphorbia pulcherima</i>	Lal patti	Euphorbiaceae	Leaves	Tannins and phenols.
8.	<i>Lantana camera</i>	Ghaneri	Verbenaceae	Leaves	Essentia oil carmine, isocamerene, micramene.
9.	<i>Mormordica charantia</i>	Karale	Cucurbitaceae	Leaves	Triterpenoids, alkaloids, charine.
10.	<i>Jatropha gossipifolia</i>	Jangali erand	Euphorbiaceae	Leaves	Tannins, phenols and flavonoids.
11.	<i>Terminalia arjuna</i>	Arjun	Combretaceae	Bark	Arjunine, Lactone, Arjunetin, tannin, phenols, flavones, alkaloids and saponin.
12.	<i>Annona squamosa</i>	Sitafal	Annonaceae	Seeds	Annonis and acetogenins.
13.	<i>Clerodendrum multiflorum</i>	Arni	Lamiaceae	Leaves	Alkaloids, flavonoids, glycosides, tannins steroids and carbohydrates.
14.	<i>Argemone mexicana</i>	Prickly poppy	Papaveraceae	Seeds	Polyphenols and polysaccharides.

Economic parameters:

In economic parameters the larval character shows the significant results. There was increase in the larval weight and larval period in plant extracts treated groups as compared to both normal and inoculated control groups.

Economic characters were studied at 6th day after the formation of cocoon. In economic characters, the silkworm inoculated with bacterial infection showed the decrease in cocoon weight, shell weight and shell ratio as compared to normal control. But in case of plant extract treated groups, there was significantly increase in cocoon weight, shell weight and shell ratio than the inoculated control but less than the normal control. Similar results were obtained in case of Fungal and Viral diseases.

In this study the results showed that, there is decrease in the mortality percentage in plant extracts treated groups and cocoon yield also high as compared to inoculated control groups of all the pathogens in both the races i.e. PM and CSR2. The cocoon weight, Shell weight and shell ratio were increased due to application of antimicrobial active plants. The plants are having phagostimulant compounds. Therefore silkworms feed with plant extracts consume the more leaves than the inoculated control and therefore significantly increase in economic parameters and also increase in the larval weight and larval duration in plant extracts treated groups in both the races.

On this study two papers are accepted:

- i) **Jyoti Ashok Chavan, Santosh Jalinder Patil and Ganesh Parashram Bhawane. (2010).** Screening of aqueous plant extracts against infection to Vth instar larvae of *Bombyx mori* L. *Journal of medicinal plant research*.
- ii) **J. A. Chavan, S. J. Patil and G. P. Bhawane. (2011).** Aqueous leaf extract of *Clerodendrum multiflorum* exhibit antifungal effect against white muscardine disease of silkworm, *Bombyx mori* L. *Bionano frontier*.

3. Biochemical study:

The total protein, carbohydrate and lipid content were estimated from normal control, inoculated control and plant extracts treated groups after three days of inoculation. In case of inoculated group there was damage in digestive system of silkworm. Therefore the protein, carbohydrate and lipid content in inoculated groups decreased as compared to normal and plant extracts treated groups. In case of plant extracts treated groups, after the treatment for three days there was elimination of pathogens from the body due to the presence of secondary metabolites in the plants like phenols, flavonoids, tannins and alkaloids etc. which are having the antimicrobial activity. In some plants there is presence of the phagostimulant compounds which effects on the larval feeding activity. After the treatment of plants extracts there was recovery in the silkworm larvae. Therefore total protein, carbohydrate and lipid content in plant extracts treated groups showed more or less similar results. In biochemical study total protein, Carbohydrate and lipid were estimated. The results showed that there is increase in protein, carbohydrate and lipid content in plant extracts treated groups as compared to inoculated groups of pathogens in both the races. There was significantly decrease in the protein, carbohydrate and lipid content in pathogen inoculated groups as compared to normal control and plant extracts treated groups.

Similarly antioxidant enzymes and Acid phosphatase, Lipid peroxidation and Non specific esterase enzyme were estimated in NPV inoculated and plant extracts treated groups. The results obtained are as follows:

Estimation of Super oxide dismutase:

The results obtained showed that SOD activity in inoculated group was decreased significantly as compared with control group. In plant extract treated groups significant increase in SOD activity was observed. The SOD activity in *C. Longa*, *A. Marmelos*, *M.*

Charantia and *S. cumini* showed highly significant increase as compared with *E. pulcherima* and *A. mexicana*.

Estimation of Catalase:

Catalase activity was increased significantly in NPV inoculated group as compared with control. In plant extract treated groups the catalase activity was decreased significantly. There was highly significant decrease in group treated with *E. pulcherima*.

Estimation of ascorbate peroxidase:

Ascorbate peroxidase activity was increased significantly in inoculated group of larvae. In case of *A. Mexicana* and *S. cumini* there was significant decrease in APx activity as compared with control group. The APx was highly increased in *A. marmelos*, *M. charantia* and *E. pulcherima* as compared with other groups.

Estimation of Acid Phosphatase:

The acid phosphatase activity was increased significantly in inoculated group as compared with control group. In case of *A. marmelos* and *S. cumini* the enzyme activity was increased with high significance. In all other plant extract treated groups the enzyme activity was not changed significantly.

Estimation of lipid peroxidation:

Lipid peroxidation activity was decreased significantly in inoculated group as compared with the control group. In case of *C. longa*, *A. marmelos*, *S. cumini* and *E. pulcherima* there was significant increase in the lipid peroxidation activity as compared with control group. In *M. charantia* and *A. mexicana* showed the decreased lipid peroxidation activity as compared to other plant extract treated groups.

Estimation of Nonspecific esterase enzyme:

The nonspecific esterase enzyme activity was increased in inoculated group as compared with control group. There was non-significant change in group treated with *S.*

cumini extract. There was low significant change in NSE activity in groups treated with *C. longa* and *E. pulcherima*. Plant extracts of *A. marmelos*, *M. charantia* and *A. mexicana* showed highly significant increase.

On this study one paper is published:

- i. G. P. Bhawane, J. A. Chavan, S. M. Gaikwad, S. R. Aland, V. J. Kumbhar and N. T. Pawar (2010). Efficacy of ethanolic plant extractives on biochemical moieties and cocoon character of *Beauveria bassiana* infected 5th instar larvae, *Bombyx mori* L. *Geobios*.37: 314-318.

4. Haematological study:

In Lepidoptera seven types of haemocytes were found. These are Granulocyte, Plasmacyte, Prohaemocytes, Spherulocyte, Coagulocyte, Oenocytoid and adipohaemocytes. The haemocytes play an important role in defense mechanism. Haemocytes are extremely effective in removing foreign particles like fungi bacteria, nematodes, and protozoa from the haemocoel by the process of phagocytosis, nodule formation or encapsulation.

In haemocyte study observations showed that the total haemocyte count was decreased on second day in inoculated groups but after the third day there was decrease in the haemocyte number as compared to normal control and plant extracts treated groups. In some plant extracts groups showed THC were more or less similar when compared with the normal control indicating that the plant extractives probably helped in the clearance of pathogen from its haemolymph.

In differential haemocyte count inoculated groups of pathogens showed increase the number of plasmacyte and granulocyte on second day for defense against the pathogen but after the third day there was decrease in number of granulocyte, plasmacytes and other cells as compared to normal control and plant extract treated

groups. Plant extracts treated groups showed the increase in the number of plasmatocyte and granulocyte as compared to normal control.

5. Neuroendocrinological study:

The results obtained in neuroendocrinology described on the basis of staining intensity of A₁ and A₂ cells of median neurosecretary cells. The stained whole mounts are observed under the microscope. The neuroendocrinological study carried out on the 5th day after the treatment of both pathogen and plant extracts.

Effects of Bacteria:

After the inoculation of bacteria Vth instar larvae of both the races get infected. The inoculated group of both races showed the A₁ and A₂ cells were stained containing moderate amount of granules of PF positive material when as compared to normal control and plant extract treated groups. The size of cells slightly increased and there is slight disruption in the cell architecture. These results indicate that there was some stress on the secretion of the activity of these cells in both the races. As compared to other pathogens the bacterial pathogens do not infect the nerve cells or neurosecretary cells of the brain. Bacteria releases the toxic substances into haemolymph through which it must be carried to brain and ventral ganglia causing paralysis like condition in the larvae. But due to the application of alcoholic plant extracts which contains the bactericide like factor which eliminate the infection of bacteria due to which there is no secretion and formation of bacterial toxins into the haemolymph. Hence there would be no toxic stress on the A₁ and A₂ cells of median neurosecretary cells of bacterial inoculated and plant extracts treated groups. Hence due to the application of alcoholic plant extracts for three days there is recovery of normal condition of silkworm.

Effects of fungus:

The white muscardine disease is caused by *Beauveria bassiana* and brown muscardine is caused by *Aspergillus flavipes*. The spores of *B. bassiana* and *A. flavipes* spread on the body of silkworm germinate and forming mycelia. The mycelia penetrate in all the organs or cells of the silkworm after the five days. The neurosecretory cells also damaged and after spreading the mycelia in the body there is no recovery of damage. Therefore on the 2nd day after the inoculation of fungal spores brain dissected and whole mount prepared and stained with PF. After the infection of *B. bassiana* and *A. flavipes* architecture of cells is disrupted and stress on the secretion of neurosecretory cells. Therefore at the time of fungal infection, after the spreading of fungal spores the silkworm larvae feed or dust with alcoholic plant extracts which having the antifungal activity. The plant extracts inhibits the germination of fungal spore therefore damage was not observed in plant extracts treated groups. But in inoculated control on the 5th or 6th day the mycelia or hyphae were also seen in the brain. Plant extracts treated groups larval brains functions normal like normal control larvae.

Effects of BmNPV:

Nuclear Polyhedrosis Virus causes the hypertrophy of nuclei of the nervous system. Tracheal innervations are the possible source for infection to Central nervous system by BmNPV. The neurosecretory cells are the modified motor nerve cells must also get infected with BmNPV due to which normal function of neurosecretion i.e. formation granules on neurosecretory material must be altered. Hence there is an effect of BmNPV infection on neurosecretory cells. Thus after infection of BmNPV there was devoid of neurosecretory cells. All the cells in nervous system get infected and total process of metamorphosis get disturbed. However the neurosecretory cells from plant extract treated groups larvae showed gradual recovery from the infection.

The silkworms of both the races i.e. PM and CSR2 treated with plant extracts for three days after the infection of bacteria, fungus and viral showed the normal behavior after the 5th day of inoculation and they were having more or less similar cocoon characters, Biochemical contents, haematological parameters and neuroendocrinological functions with normal control silkworm larvae. The pathogen inoculated larvae after treatment of plant extract shows the normal feeding like control group and hence they recovered from infection of bacteria, fungus and viral infection.

The pathogenic infection of bacteria, fungus and viral to silkworm, *Bombyx mori* L. is very common and resulting into the known disease i.e. flacherie, muscardine and grasserie which causing total failure of many crops in the past and certain crops in particular season in particular geographical area even in the present day due to non adoption of standard practices of rearing, scheduled of disinfection and prevailing favourable climatic condition in the microclimate of rearing house. Recently in sericulture practices along with preventive measures, routine use of disinfectants in the disease management of silkworm diseases must be carried out. Also importance is given to the curative measures where use of certain antibiotics and other preparations were recommended. Now a days the use of herbal preparation as they are natural products are gaining importance in variety of purpose like medicine, insecticides, juvenoid, growth promoters, phagostimulants, disinfectants, antioxidants, antibacterial, antifungal and antiviral agents due to the presence of secondary metabolites like phenols, polyphenols, tannins, flavonoides, terpenes etc. also present in the plants.

Hence in the project undertaken efforts are made in screening of herbs for their antimicrobial activities and results as mentioned above are quite encouraging. Secondly the mode of application of plant extractives as antimicrobial agent in the silkworm disease management is cumbersome and not farmer friendly as it is given in the form of

aqueous solution by leaf dipping method. Hence simple methodology of application like dusting of larvae along with lime carrier or other is to be evolved individually or in appropriate combination of different plant products used in the screening.

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