ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 5, Issue, 07, pp.1709-1711, July, 2013

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

IMPACT OF PLANT EXTRACTS ON BIOCHEMICAL CHANGES IN *Bacillus thuringiensis* INFECTED SILKWORM, *Bombyx mori* L.

***Cahvan, J. A. and Bhawane, G. P.**

Department of Zoology, Shivaji University, Kolhapur- 416-004

ARTICLE INFO

Article History:
Received 23th April, 2013
Received in revised form
29th May, 2013
Accepted 10th June, 2013
Published online 18th July, 2013

Key words:
Bombyx mori, BmNPV, *Aegel marmelos, Curcuma lounga.*

ABSTRACT

Nickel In the present investigation the effect of plant extracts on biochemical changes in *Bacillus thuringiensis* infected fifth instar silkworm larvae were studied. The changes in the absorption, digestion and transportation of nutrients in the mid gut of silkworm.

INTRODUCTION

Mulberry silkworm, *Bombyx mori* L. being an economically important insect, is under domestication from the past 4000 years. Silkworm diseases are the major problem in sericulture industry. Among the silkworm diseases flacherie disease causing serious problem in mass rearing of silkworm in tropical countries like India. Flacherie is more common in fourth and fifth instar during all season causing 20 to 50 % cocoon crop losses in India (Natraju *et al*.,1998; Samson *et al*., 1990). Bacterial flacherie also known as "Bacterial diseases of digestive organ" is caused due to the multiplication of different kinds of bacteria like *Streptococci, E. coli,* Aerogenous *bacilli* and proties group *Bacilli* in the alimentary canal, disturbing the normal function of the gut, multiply in the digestive tract and destroy the membranous tissue. Bacterial toxicosis (sotto disease) is caused by different strains of *Bacillus thuringiensis.* This disease transmitted through oral ingestion of the spores or the crystalline parasporal body along with the mulberry leaves their spores produce toxic substances, which dissolve in the alkaline gut juice and are absorbed through the gastric wall affecting the nervous system to spasm and paralysis. The toxic material changes the pH of the haemolymph from 9.0 to 10.5 (Aruga, 1994).

Effect of *Bacillus thuringiensis* and *Serratia marcescens* on the alkaline phosphatase activity in the mid gut of silkworm has been investigated (Miao and Bharathi, 2001). Microscopic examination reveals that the epithelial cells are disrupted and may eventually breakdown completely (Nishiitsutsuji-Uwo and Endo, 1979). The histopathological changes in the mid gut epithelium of *B. mori* following administration of delta-endotoxin in vivo was studied (Endo and Nishiitsutsuji-Uwo, 1979). The effect of *Bacillus thuringiensis* on ultra structural studies of silkworm mid gut was studied (Percy and Fast, 1983). The AIKP was related to the physiological situation of silkworm and reflects on the absorption, digestion and positive transportation of nutrients in the mid gut (Eguchi and Iwamoto, 1975 and Eguchi *et al*.,1990; Yoshitake, 1966). Plant extract have greater potential as antimicrobial compounds against microorganism. Thus, they can be used in treatment of infectious disease caused by resistant microbes. The flavonoid rich extracts prepared from *E. teriticornis* exhibited antifungal and antibacterial activity (Barnabas and Nagarajan, 1988). Plants are contain several classes of secondary metabolites like simple phenols, phenolic acids, quinines, flavones, flavonoides and flavonols, tannins, coumarins, terpenoides and essential oils. Terpenenes or terpenoides are active against bacteria (Amaral *et al.,* 998; Hambuger *et al.,*1999; and Kubo *et al.,* 1995). In the present investigation the effect of plant extracts on biochemical changes in *bacillus thuringiensis* infected fifth instar *B. mori* L. were studied.

Corresponding author: Cahvan, J. A.
Department of Zoology, Shivaji University, Kolhapur- 416-004

MATERIAL AND METHODS

Experimental animals

The egg layings of popular multivoltine race, Pure Mysore (PM), supplied by Directorate, Government Sericulture Grainage Center, Suleran, Dist-Kolhapur, Maharashtra, India for laboratory rearing. The larvae were reared as per the method of Krishnaswamy (1978, 1979). *B. thuringiensis culture* made available from M.T.C.C. Institute of Microbial Technology, Chandigarh-160036 India. Pure culture were maintained as per the procedures of Govindan *et al.,* (1998).

Treatment of pathogen and plant extract to B. mori

In case of bacteria, freshly collected leaves of mulberry (50g) deeped in 50 ml of LC_{50} concentration of bacterial suspension. The leaves are dried at room temperature and given to freshly moulted and two hours starved 5th instar larvae. After six hours of treatment the larvae were segregated to appropriate groups for giving treatment of plant extracts. The plants selected for present study was *Aegel marmelos* and *Curcuma lounga* which are having antimicrobial activity. The plant extractx are given along with the mulberry leaves. 50 g leaves deeped in 4000ppm concentration of extracts and shade-dried before feeding to larvae. treatment is given for three days during morning

feed only. Total protein (Lowry *et al.,* 1951), total lipid (Barnes and Blackstock, 1973) and carbohydrate (Noelting and Bernfeld, 1948). Were estimated in the normal control, inoculated control and plant extract treated groups.

RESULTS

The data presented in the Table 1. reveal the extent of biochemical changes in normal, inoculated and plant extract treated groups of silkworm, *B. mori* L.

Table 1. Biochemical mioties

S.No.	Groups	Carbohydrate mg/g	Protein mg/g	Lipid mg/g
1.	Normal Control	1.778 ± 0.123	290.6 ± 0.178	178.0 ± 0.167
2.	Inoculated Contro	0.998 ± 0.99	172.4 ± 0.111	118.0 ± 0.179
3.	*Aegel marmelos*	1.348 ± 0.212	228.0 ± 0.089	166.0 ± 0.998
4.	*Curcuma longa*	1.212 ± 0.312	191.2 ± 0.231	162.7 ± 0.562

Carbohydrate content

There was significantly increase in carbohydrate content after the treatment of plant extract than the inoculated control but not than the normal control. The normal control larvae contain 1.778 mg/g of tissue of carbohydrate content and inoculated control contain 0.998 mg/g. The *A. marmelos* and *C. lounga* treated group of larvae contain the carbohydrate content 1.348 and 1.212 mg/g respectively.

Protein content

The protein content in the inoculated control was decreased 172.4 mg/g than the normal control 290.6 mg/g and the plant extract treated groups *A. marmelos* 228.0 mg/g and *C. lounga* 191.2 mg/g.

Lipid content

There was increasing the lipid content after the treatment of plant extracts of *A. marmelos* 166.0 mg/g and *C. lounga* 162.7 mg/g than the inoculated control 118.0 mg/g but not than the normal control 178.0 mg/g.

DISCUSSION

The results revealed that the larvae infected with *B. thuringiensis* showed the decrease in biochemical content but the plant extract treated groups were effective in reducing disease incidence. In *B. mori* inoculated control showed the decrease in carbohydrate, protein and lipid content because the *B.thuringiensis* caused damage to the epithelial cells of the mid gut through crystalline parasporal body which could release toxic parasporal crystals under alkaline condition in the intestinal juice (Endo and Nishiitsutsuji, 1980; Percy and Fast, 1983 and Mathavan *et al.,* 1989). *B. thuringiensis* are the indicators of general cell injury or necrosis and are attributable to specific toxic action of crystal toxin (Percy and Fast, 1983). The decrease in the carbohydrate, protein and lipid content in inoculated control might be due to pathological symptoms such as disruption of epithelial cells of mid gut (Nishiitsutsuji and Endo, 1979). At the present natural botanicals are being exploited in the area of plant and animal disease and pest management. These are being used in silkworm rearing to reduce disease or to improve cocoon yield. Allelochemicals from plats have deleterious effect on disease causing pathogen of insects and the insects ingesting such allelochemicals could survive infections better (Beranhaum, 1988). Samuel Manohar Raj (1994) reported that aqueous extract of *Psoralea coryleifolia* and *Tribulus terrestris* suppressed the grassarie disease in silkworms. Barnabas and Nagarajan (1988) have reported that the flavonoid rich extracts prepared from *E. teriticornis* exhibited antifungal and antibacterial activity against several test organisms including *Aspergillus flavus* and *Candida albicum.* According to Jeyapaul *et al.* (2003), plant extracts exhibited growth stimulating action and can be used to increase the cocoon weight. Murugan *et al.* (1994) observed that extrafoliation of aqueous extract of leaves of *Tribulus terrestris* L. and *Phyllanthus niruri* L. increased the total body weight, silkgland weight and silk filament length. Chopra *et al.* (1956) observed that the *A. marmelos* fruits having the antibacterial activity and Cowan (1999) reported that the *C. lounga* rhizome having antibacterial activity. Hence it can be concluded that the larvae infected with *B. thuringiensis* showed the action of toxin of the cells of mid gut was extremely rapid which affect the assimilatory capacity in the alimentary tract and biosynthetic activities in the body and general metabolic breakdown occurs. Thereby the growth of silkworm was affected which might be due to physiological damage but application of plant extracts of *A. marmelos* and *C. lounga* reduces the disease incidence and improve the carbohydrate, protein and lipid content than the inoculated control not than the normal control. All these reports confirms that the present study with respect to disease reducing properties of plants of *A. marmelos* and *C. lounga*. Therefore both the plants are useful to silkworm disease management and benefiting to farmers by saving their crops.

Acknowledgments

Authors are thankful to Head Department of Zoology, Shivaji University, Kolhapur for providing laboratory and other infrastructure facilities.

REFERENCES

Amaral, J. A, Ekins, A., Richards, S. R. 1998. Knowels R. Effect of selected monoterpenes on methane oxidation, denitrification, and aerobic metabolism by bacteria in pure culture. Appl. Environ. Microbiol., 64: 520-525.

Aruga, H. (1994). Principles of sericulture (Transform Japanese). Oxford and IBH publishing Co. Pvt. Ltd. New Delhi, India.

Barnabas, C. G. and Nagarajan, S. 1988. Antimicrobial activity of flavonoids of some medicinal plants, Fitoterapia, 59 (6): 508-510.

Barnes, H. and Blackstock, J. Estimation of lipids in marine animals, details investigation of the sulphophosphovanillin method. J. Exp. Man. Biol. Ecol., 1973, 12, 103-108.

Beranhaum, M. R. 1988. Allelochemicals in insect microbe plant interactions, against provocatears in the co-evolutionary process. In: Novel Aspects of Insect Plant Interaction, Barbaso, P. and Leteurmeau, D. K. (Eds.) p. 97-123.

Chopra, R. N. and S. L. Nayer, 1956. Glossary of Indian Medicinal plants, India: Dera Dun publisher Ltd., pp:117.

Cowan, M. M. 1999. Plant products as antimicrobial agents. Clinical Microbiology Revies, 12 (4) p. 564-582.

Eguchi, M and Iwamoto, A., 1975. Changes in protease, esterase and phosphatase in the alimentary canal of the Silkworm during metamorphosis. Insect biochem., 5:495-507.

Eguchi, M., Azuma M., Yamamoto H., and Takeda S., 1990.Genetically defined membrane bound and soluble alkaline phosphate of the Silkworm : their discrete localization and properties. Isozymes : structure, function and use in biology and medicine, Wiley-liss Inc., New York. Pp.267.

Endo, Y. and Nishiitsutsuji-Uwo, J., 1979. Ultra structural changes in the mid gut epithelium of *Bombyx mori* induced by *Bacillus thuringiensis* crystals. Japanese J. Zool. 23:183-185.

Endo, Y., Nishiitsutsuji-Uwo, J., 1980. Mode of action of *Bacillus thuringiensis* δ endotoxin : Histopathological changes in the Silkworm midgut. J. Invert. Pathol., 36: 90-103.

Govindan, R., Narayanswamy, T. K. and Devaiah, M. C. *Principles of Silkworm Pathology*. SERI Scintific Publishers, Bangalore, 1998.

Hamburger, H. and Hostettmann, K. (1991). The link between phytochemistry and medicine. Phytochemistry. 30: 3864-3874.

Jeyapaul, C., Padmalata, C., Rajit Singh, A. J. A., Murugesan, A. G. and Dhasarathan P. 2003. Effect of plant extract on nutritional efficiency in mulberrr Silkworm *Bombyx mori* L. Indian J. Seric., 34(2) 128-131.

Krishnaswamy, S. Improved method of rearing young age silkworm, CSR and TI Bulletin, 1979, 3, 1-24.

Krishnaswamy, S. *New Technology of Silkworm Rearing*. CSR and TI Bulletin, 1978, 2, 1-23.

Kubo, I., Muroi, H., Himejma, M. (1995). Antimicrobial activity of totarol and its potentiation. J. Nat. Prod., 55: 1436-1440.

Lowry, O. H., Roserbrough, N. J., Farra, L. and Randall, R. J. Protein measurement with the folin phenol reagent. J. Biol. Chem., 1951, 193, 265-275.

Mathavan, S., Sudha, P.M. and pechimuthu, S.M., 1989.Effect of *Bacillus thuringiensis* israelensis on the midgut cells of *Bombyx mori* larvae: A histopathological and histochemical study. J. Invertbr. Pathol., 53 (2), 217-227.

Miao, Y. and Bharathi, D., 2001. Effect of *Bacillus thuringiensis* and *Serractia marcescence* on the mid gut of Silkworm, *Bombyx mori* L. *Ind. J. Seric.* 40:103-105.

Murugan, K., Jeyabalan, D., Senthil kumar, N., Sivaramakrishna, S., and Sivaprakasam, N. 1999. Growth promoting effect of plant compounds on Silkworm, procceding of "National Seminar On Tropical Sericulture" held during 28-30th December. P.107-108.

Nataraju, B. Datta, R. K., Baig, M., Balavenkatasubbaiah, M., Samson, M.V., and Shivprasad, V. 1998. Studies on the prevalence of NPV in sericulture area of Karnataka. Indian J. Seric., 37 (2): 154-158.

Nishiitsutsuji-Uwo, J and Endo, Y., 1979. Mode of action of *Bacillus thuringiensis.* δ-endo toxin: Effect on TN-368 cells. J. Invert. Pathol. 34: 267-275.

Noelting, G. and Bernfield, D. Sur les enzyme amylolytigues III La β- amylase dosage diactive et control de absence amylase. Helv. Chim. Acta, 1948, 31, 286-290.

Percy, J. and Fast, P. G., 1983. *Bacillus thuringiensis* crystals toxin : Ultra structural studies of its effect on Silkworm mid gut cells. J. Invert. Pathol. 41: 86-88.

Samson, M. V., Baig, M., Sharma, S. D., Balavenkatasubbaiah, M., Sasidharan, T.O. and Jolly M. S. 1990. Survey on the relative incidence of silkworm disease in Karnataka, India. Indian J. Seric., 29 (2): 248-254.

Samuel Manohar Raj, K. 1994. Effect of certain botanicals on the nuclear polyhedrosis virus disease of Silkworm, *Bombyx mori* L. M.Sc. Dessertation, Tamil Nadu Agricultural University, Coimbatore, India, P.62.

Yoshitake, N., Eguchi, M. and Akiyama., 1966. Genetic control on the alkaline phosphtase of the mid gut in Silkworm, *Bombyx mori* L. J. Seric. Sci.japan. 35: 1-6.

****