Antifungal effect of *Ocimum sanctum* L. against white muscardine disease of silkworm, *Bombyx mori* L.

P. Padma Sree Vidya Devi and M. Ramani Bai*

ABSTRACT

The **"**Queen of Herb**",** Tulsi is an important herb with greater medicinal value used in Ayurveda, Siddha, Unani, Greek and Roman medicine for prevention and cure of many illness. Fifth instar larvae inoculated with *Beauveria bassiana* were fed with mulberry leaves enriched with different concentrations (1 %, 2%, 3% and 4%) of ethanolic extract of *Ocimum sanctum* leaves and its effect on cocoon weight, pupal weight, shell weight, shell ratio and silk characteristics were studied. Among the different concentrations, 3% ethanolic extract of *O. sanctum* has been found to be most effective against *B. bassiana* infection as compared to others. Inoculated control group recorded very low economic values as compared to normal control and other treated groups.

MS History: .25.09.2014 (Received)-08.11.2014 (Revised)-18.11.2014 (Accepted)

Citation: P. Padma Sree Vidya Devi and M. Ramani Bai. 2014. Antifungal effect of *Ocimum sanctum* L. against white muscardine disease of silkworm, *Bombyx mori* L. *Journal of Biopesticides*, 7(2):204-208.

Key words: Mulberry, *Ocimum sanctum, Beauveria bassiana, Bombyx mori*, cocoon character.

INTRODUCTION

The domesticated silkworm, *Bombyx mori* L. being an insect of commercial importance, has been a target of intensive scientific study for a long time for the development of sericulture technology in silk producing countries. It is affected by a variety of pathogens such as, fungi, bacteria, viruses and protozoans, which cause several diseases leading to cocoon crop loss, ultimately affecting the whole sericulture industry. Among the fungal diseases, white muscardine caused by a pathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin (1912) is a common and widely prevalent disease affecting *B. mori* in all the countries practicing sericulture especially during the rainy and winter seasons. *B. bassiana* infects the larval and pupal stages of the mulberry silkworm, *B. mori* (Kumar *et al.,* 2013). **"**Prevention is better than cure**"** is the most suitable proverb for silkworm diseases because there are no curative methods for the silkworm diseases including white muscardine.

The plant derived compounds contribute to a great extent in fight against pathogenic microorganisms (Vyvyan, 2002).The efficacy of several fungicidal formulations (Krishnaprasad *et al.,* 1978), bed disinfectants (Bhattacharya *et al.,* 1995), antifungal chemicals (Amutha *et al*., 2010), herbal extracts (Isaiarasu *et al*., 2011) and botanicals (Chavan *et al.,* 2011 a, b and 2013), against *B. bassiana* had been already evaluated. *O. sanctum* is an important medicinal herb. Hence the present study was made to find out the antifungal effect of *O. sanctum* plant extract against white muscardine disease of silkworm, *B. mori.*

MATERIALS AND METHODS

Rearing of *B.mori*

The disease free layings of PM x CSR_2hybrid silkworm, *B. mori* was procured from the Government Grainage Center, Konam, Nagercoil. The silkworm larvae were reared as per rearing method of Krishnaswami (1978).

Fungus culture

The fungus culture of *B. bassiana* was obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India. The fungus culture was maintained as per the procedure of Govindan *et al*. (1998).

Plant extract preparation

The fresh *O. sanctum* leaves were collected from areas around Nagercoil. The leaves were washed and shade dried at room temperature and powdered in a mechanical grinder. 10 g of air dried powder was placed in 100 ml of ethanol in a conical flask and kept in rotary shaker at150 rpm for 24 hrs. After 24 hrs, it was filtered by using Whatmans filter paper No.1 and the solvent was evaporated. The paste extract was stored at 4^0C until further use.

Treatments against *B. mori*

LD_{50} values are important to evaluate the toxicity level and allow the determination of the sub- lethal doses. The present study began with the determination of 96 hrs-LD_{50} of *B. bassiana* to PMxCSR2.The treatment of LD_{50} concentration of *B. bassiana* was given to freshly moulted and two hours starved fifth instar silkworm larvae. After six hours of pathogenic treatments, the larvae were fed with ethanolic extract of *O. sanctum* at different concentrations such as; 1%, 2%, 3% and4% to appropriate groups. The treated mulberry leaves were shade dried before providing to larvae. The treatment was repeated for three days in the morning feed only. One group was fed with pathogen treated mulberry leaves (inoculated control).The normal control group was fed with fresh mulberry leaves without any application. Experimental as well as control group has three replications consisting of 50 larvae each. Economic parameters such as, cocoon weight (mg), pupal weight (mg), shell weight (mg), shell ratio (%) and silk characters like fibroin content (mg), sericin content (mg), filament length (m) and denier were recorded andanalysed statistically to study the effect (Zar,1984).

RESULTS AND DISCUSSIONS

The results indicates the impact of *B. bassiana* and plant extract on economic characters of *B. mori*. Among different concentrations of *O. sanctum* (1%, 2%, 3% and 4%) treated mulberry leaves fed to inoculated fifth instar silkworm larvae, the average cocoon weight was found highest in the 3% concentration (1081.12±49.88 mg), followed by 4% (1027.14±43.98mg), 2% (954.46±27.43mg), 1% (886.16±14.11mg) and inoculated control (725.24±14.34mg).The shell weight was increased by 116.70 per cent compared to inoculated control (Table1.)

Table 1. Effect of *O. sanctum* on the cocoon characters of *B. bassiana* infected silkworm, *B. mori*.

Treatments	Concentrations	Cocoon weight (mg)	Pupal weight (mg)	Shell weight (mg)	Shell ratio (%)
Plant extract +*B.bassiana*	1%	886.16±14.11 * (-19.90) **(22.19)	780.10±12.75 *(-15.60) **(19.62)	105.25±14.82 *(-42.25) **(43.28)	11.85±1.11 * (- 27.79) **(1.79)
	2%	954.46±27.43 *(-13.73) **(31.61)	825.24±14.71 *(-10.71) **(26.54)	128.17±16.41 *(-29.67) **(74.48)	13.41±1.09 *(-18.8) **(32.90)
	3%	1081.12±49.88 *(-2.28) **(49.07)	921.11±57.55 *(-0.34) **(41.24)	159.24±18.14 *(-12.62) **(116.70)	14.72±1.37 *(-10.30) **(46.32)
	4%	1027.14±43.98*(-7.16) **(41.63)	881.18±38.16 *(-4.66) **(35.12)	145.46±17.76 *(-20.18) **(98.01)	14.11±1.34 *(-14.02) **(40.26)
Inoculated control	-	725.24 ± 14.34 *(-34.45)	652.16±12.41 *(-29.24)	73.46 ±22.53 *(-59.69)	10.06±1.02 *(-38.70)
Normal control	-	1106.40±106.1	924.26±85.48	182.24±20.76	16.41±1.48

* Values in parentheses indicate the percentage change over the normal control; ** Values in parentheses indicate the percentage change over the inoculated control.

Table 2. Effect of *O. Sanctum* on the silk characters of *B. bassiana* infected silkworm, *B. mori*.

Treatments	Concentrations	Fibroin (mg)	Sericin (mg)	Filament length (m)	Denier
Plant extract +*B.bassiana*	1%	63.12±6.09 *(-38.60) **(49.68)	31.48±4.04 *(-55.18) **(53.56)	308.00±36.11 *(-31.89) **(7.26)	1.16±2.14 *(-29.27) **(26.08)
	2%	75.00±7.08 *(-27.04) **(77.85)	41.50±5.83 *(-40.91) **(102.4)	.00±12.17 *(-24.37) **(19.11)	1.32±2.06 *(-19.51) **(43.47)
	3%	90.46±8.99 *(-12.00) **(114.2)	58.23±10.22 *(-17.09) **(184.0)	419.00±13.21 *(-7.34) ** (45.92)	1.56±3.28 *(-4.88) **(69.56)
	4%	86.14±7.02 *(-16.21) **(104.7)	47.72±7.32 *(-32.05) **(132.7)	396.10±23.12 *(-12.40) **(28.54)	1.43±2.58 *(-12.80) **(55.43)
Inoculated control	-	42.17±6.43 *(-58.98)	20.50 ±4.53 *(-70.81)	287.14±10.32 *(-36.50)	0.92±1.08 *(-43.90)
Normal control	-	102.8±10.22	70.23± 7.32	452.18±12.34	1.64±3.54

* Values in parentheses indicate the percentage change over the normal control; ** Values in parentheses indicate the percentage change over the inoculated control.

Manimegalai *et al.* (2010) suggested that natural plant products are used to control various pathogenic diseases of silkworm. Medicinal plants represent a rich source of antimicrobial agents. Kishore *et al*. (1982) reported that the extract of *O. sanctum* contains eugenol that has antimicrobial activity. Mahesh and Satish (2008) revealed that plants generally produce many secondary metabolities which constitute an important source of microbiocides and pesticides. Recently several reports have come on antimicrobial activity of plant extracts against bacteria and fungi (Mohanan *et al*., 2007 and Chavan *et al*., 2011b). Ethanolic extract was reported to be a better solvent for extracting the antimicrobial active substances compared to other solvents (Ahmad *et al.*,1998).The results revealed that ethanolic leaf extract of *O. sanctum* was effective in controlling white muscardine disease caused by *B. bassiana* and gained the cocoon weight, pupal weight, shell weight and shell ratio. Jayapaul *et al*. (2003) investigated that the *B. mori* larvae fed with *Coffea arabica* leaf extracts treated mulberry leaves, recorded higher shell weight. Murugesh and Mahalingam (2005) reported that *Tribulous terrestris* leaf extract improved the cocoon characters of silkworm, *B. mori*. According to Chavan *et al*. (2013), the ethanolic extract of *Clerodendrum multiflorum* can be used to increase the cocoon parameters and is in agreement with the present report.

Significant higher silk characteristics such as; fibroin and sericin contents, filament length and denier were observed in all the plant extract treated groups except in inoculated groups. Fibroin content was found to be the highest in the 3% concentration (90.46±8.99 mg) over the inoculated control (42.17± 6.43 mg).Filament length was increased by 45.92 per cent and denier by 69.50 per cent when compared to their respective inoculated controls (Table 2).Gouda (1991) recorded that *Psoralea coryleifolia* extract improved the silk characters of silkworm, *B. mori*. According to Sujatha and Rao (2004) the lower concentration of tulsi and neem powder not only reduced disease but also enhanced the economic characters of *B. mori.* As per Sangamithirai *et al*. (2014) treatment of *Spirulina* at the concentration of 300ppm had beneficial effects on the quantitative parameters of silkworm, *B. mori.* Pardesh and Bajad (2014a and b) reported that the moderate concentration (2.5%) of *Xanthium indicum* L. and *Amaranthus hybridus* extracts had beneficial effect on economic parameters of silkworm, *B. mori.* Accordingly, the present study indicates that 3% *O. sanctum* leaf

extract can be used effectively for the management of white muscardine disease in silkworm, *B. mori*, which inturn could improves the cocoon and silk yield.

REFERENCES

Ahmad, I., Mehmood, Z. and Mohammad, F.1998. Screening of some Indian medicinal plants for their antimicrobial properties. *Journal of Ethnopharmacol*, **62** (2): 183-193.

Amutha, M., Banu, J.G., Surulivelu, J. and Gopalakrishnans, N. 2010. Effect of commonly used insecticides on the growth of white muscardine fungus, *Beauveria bassiana* under laboratory conditions. *Journal of Biopesticides*, **3(**1): 143-146.

Bhattaacharya, J. N., Krishnan, A. K., Chandra, S.K., Sen and B. Saratchandra, 1995. LABEX: An effective bed disinfectant. *Indian Silk*, **34:**35-36.

Chavan, J.A., Patil, S.J. and Bhawane, G. P.2011 a. Screening of aqueous plant extracts against *Beauveria bassiana* infection to V instar larvae of *Bombyx mori* L. *Journal of Medicinal Plants Research*, **5**(16): 3936-3939.

Chavan, J.A., Patil, S.J. and Bhawane, G. P. 2011b. Aqueous leaf extract of *Clerodendrum multiflorum* exhibit antifungal effect against white muscardine disease of silkworm, *Bombyx mori* L. *Bionano Frontior*,**4**(2) : 247-249.

Chavan, J.A., Patil, S.J and Bhawane, G. P.2013. Effect on the cocoon character of *Beauveria bassiana* infection and subsequent treatment of ethanolic plant extracts on V^{th} instar larvae of *Bombyx mori* L. *Journal of Science and Technology*, **21** (1):45-52.

Gouda, R.1991. Studies on methods of increase silk yield of *Bombyx mori* L. Ph.D. Thesis, Tamil Nadu, Agricultural University, Coimbatore, India, **242 P.**

Govindan, T.K., Narayanaswami, T.K. and Devaiah, M. C. 1998. "Text book of Principles of silkworm Pathology" Seri Scientific Publishers, Bangalore, **239 P.**

Isaiarasu, L., Sakthivel, N., Ravikumar, J. and Samuthiravelu, P. 2011. Effect of herbal extracts on the microbial pathogens causing flacherie and muscardine diseases in the mulberry silkworm, *Bombyx mori* L. *Journal of Biopesticides*, **4**(2): 150-155.

Jayapaul, C., Padmalatha, C., Rajitsing, A.J.A., Murugesan, A.G. and Dhasarathan, 2003.Effect of plant extracts on nutritional efficiency in mulberry silkworm, *Bombyx mori* L. *Indian Journal of Sericulture*, **34** (2):128-131.

Kishore, N., Dubey, N.E., Triathi, R.D. and Singh, K.1982. Fung / toxic activity of leaves of some higher plants. *National Academy of Science Letter*,**5**(1): 9-12.

Krishnaprasad, K.S., Siddaramaiah, A.L. and Srikanth, K.1978. Laboratory evaluation of bavistin against muscardine disease. *Indian Journal of Sericulture*, **17**(1): 69.

Krishnaswami, S. 1978. New technology of silkworm rearing .C.S.R.I. and J.I. Central Silk Board, *India. Bulletin*, **2**: 1-23.

Kumar,V., Singh, G.P., Babu, A.M., Ahsan, M. H. and Datta R.K. 2013.Germination, penetration and invasion of *Beauveria bassiana* on silkworm, *Bombyx mori* L. causing white muscardine. *Italian Journal of Zoology*, **66**(1): 39-42.

Mahesh, B. and Satish, S. 2008. Antimicrobial activity of some important medicinal plants against plant and human pathogens. *World Journal of Agricultural Science*, **4**(5): 843.

Manimegalai, S., Rajeswari, T., Shanmugam, R. and Rajalakshmi, G. 2010. Botanicals against nuclear polyhedrosis virus infecting three breeds of mulberry silkworm, *Bombyx mori* L. *Journal of Biopesticide*, **3**(1) : 242-245.

Mohanan, N.M., Guta, S.K. and Mitra, P. 2007. Antimicotic activity of *Allium sativum* against *B. bassiana*, pathogenic fungus of white muscardine disease in silkworm, *Bombyx mori* L. *International Journal of Industrial Entomology*,**14**(2):81-85.

Murugesh, K. and Mahalingam, I. 2005. Influence of *Tribulous terrestris* L. on the growth of silkworm, *Bombyx mori* L. and its impact on economic traits. In; Progress of research in organic sericulture and seri –

by products utilization, University of Madras, Vellore, 151-155M **P**.

Pardesh, A.B and Bajad, P.N. 2014(a). Effect of *Xanthium indicum* L. plant extract on the economic parameters of silkworm, *Bombyx mori* L. *International Journal of Recent Scientific Research*,**5**(3): 683-686.

Pardesh, A.B. and Bajad, P.N. 2014(b). The effect of nutritional supplementation with *Amaranthus hybridus* L. extract on economic performance of mulberry silkworm, *Bombyx mori* L. *School of Academic Journal in Bioscience*, **2** (4): 272-276.

Sangamithirai, V.N., Sabhanayagam, S., Susithra, N., Ganeshprabhu, P. and Mathivanan, V. 2014. Studies on the quantitative parameters of silkworm, *Bombyx mori* L. fed with control and *Spirulina* treated MR2 mulberry leaves. *International Journal of Modern Research and Review*, **2**(2): 79-82.

Sujatha, K. and Rao, A.P. 2004. Efficacy of certain botanicals against disease and post cocoon characters of silkworm, *Bombyx mori L. International Journal of Experimental Zoology in India*, **7**(2): 229-235.

Vyvyan, J.R. 2002. Allelochemicals as leads for new herbicides and agrochemicals. *Tetrahedron*, **58**: 1631-1646.

Zar, J.H. 1984. Bio statistical Analysis.Second edition, Prentice Hall, Engle wood cliffs, **718 P.**

P. Padma Sree Vidya Devi and M. Ramani Bai*

Department of Zoology and Research Centre, Scott Christian College (Autonomous), Nagercoil-629003, Tamil Nadu, India.

***Communication author

Email: ramanibaisjs @ gmail.com