Int. J. Adv. Res. Biol. Sci. (2023). 10(12): 30-36

International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 10, Issue12-2023

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2023.10.12.004

Study of bacterial sotto disease (*Bacillus thuringiensis*) infestation during rearing of mulberry silkworm in different meteorology

Sanjai Kumar Gupta[1], Vikas Singh[2], Ram Pravesh[3] & Purshottam Yadav[4]

[1]Assistant Professor Zoology, Department of Zoology, Silkworm/Entomology Laboratory, Veer Bahadur Singh Post Graduate Government Degree College, Campierganj, Gorakhpur-273158 (INDIA)

[2, 3, 4]Research scholar at Department of Zoology, Silkworm/Entomology Laboratory, Government Model Degree College, Barakhal, Santkabirnagar-272271(INDIA)

Corresponding Author: *drsanjaigupta1976@gmail.com*

Abstract

Silkworms are damaged by several disease insect pests like sotto disease (*Bacillus thuringiensis*). Sotto disease is an economically important endo larval parasitoid that causes yield loss of silk. Present investigation was carried out during meteorology of summer and rainy under laboratory in BOD incubator. Rearing of multivoltine hybrid Pure Mysore in different meteorology reveals that rainy meteorology open rearing is found to be susceptible. Longevity of the *Bacillus thuringiensis* is reported to be negatively correlated with meteorology parameters such as temperature; higher the temperature lowers the adult longevity. Experimental observation revealed that rearing of multivoltine Pure Mysore silkworm in summer meteorology with all the precautionary measures under protection is suitable for more yield of cocoon with least bacterial disease *Bacillus thuringiensis* infestation in all over India.

Keywords: *Bacillus thuringiensis*, multivoltine races, infestation, damage, cocoon, mulberry, rainy season meteorology, summer meteorology, open rearing and protected rearing.

ARTICLE INFO

Article History:

Received 27th November, 2023
Received in revised form 12th December, 2023
Accepted 21st December , 2023
Published online 31st December, 2023

Introduction

India is the second largest producer of silk in the world with the unique distinction of being the only country producing all the five known commercial silks. There are several factors that influence silk production, in which insect and non-insect pests is the important component and, among the insects that attack silkworm. *Bacillus thuringiensis* is soil dwelling bacterium that naturally produces a toxin that is fatal to herbivorous silkworm insect. The toxic produced by *Bacillus thuringiensis* has been used as insecticide spray since the 1920 and is commonly used in organic farming of mulberry garden. *Bacillus thuringiensis* is also the source of the genes used to genetically modify a number of food crops so that they produce the toxin on their own deter various insect pests. The toxin is lethal to several orders of insects including Lepidoptera though a number of *Bacillus thuringiensis* stains are available to make its use more targets specific. Susceptible vigorous feeding must ingest toxin crystals in order to be affected the larval life. In contrast to poisonous insecticides that target the nervous system, *Bacillus thuringiensis* toxin acts by producing a protein that blocks the digestive system of the insect, effecting starving it, toxin is a fast acting insecticides and infected insect will stop feeding within hours of infestation of mulberry leaves and infected insect will die generally from starvation or a rupture of the digestive system within day. Individual Cry toxin and Bt toxin in *Bacillus thuringiensis* has a defined spectrum of insecticidal activity, usually restricted to a few species in one particular order of Lepidoptera (butterflies and moths of silkworm), **(De Maagd, R. A. 2001 and Marroquin, et al. 2000).** A few toxins have an activity spectrum that spans two or three insect orders. For example, Cry1Ba and Bt. toxin are most notably active against the larvae of silk moths, flies, and beetles **(Zhong, 2000).** The combination of toxins in a given strain, therefore, defines the activity spectrum of that strain. Most δ-endotoxins are encoded by crygenes. The toxins were originally classified into four classes according to their amino acid sequence homology and insecticidal specificities **(Hofte, et. al. 1989).** CryI toxins are toxic to lepidopteron; CryIIs are toxic to lepidopteron and dipterans; CryIIIs are toxic to coleopterans; CryIVs are toxic to dipterans. Two additional classes, CryV and CryVI, were added for the nematode-active toxins **(Feitelson, et. al. 1992).** Currently, the toxins are classified only on the basis of amino acid sequence homology, where each protoxin acquired a name consisting of the mnemonic Cry (or Cyt) and four hierarchical ranks consisting of numbers (e.g., Cry25Aa1), depending on its place in a phylogenetic tree **(Crickmore, et. al. 1998).** Thus, proteins with less than 45% sequence identity differ in primary rank (Cry1, Cry2, etc.), and 78% and 95% identity constitute the borders for secondary and tertiary rank, respectively. In this context, the present investigation was carried out to evaluate the incidence of *Bacillus thuringiensis* sotto bacterial disease in different meteorology under laboratory conditions which affect the broad spectrum of primarily leaf-feeding lepidopteron targets.

Materials and Methods

The experiment was conducted on *Bacillus thuringiensis* to study the infestation and damage caused by *Bacillus thuringiensis* for silkworm rearing during two different meteorology i.e., summer and rainy meteorology. The freshly egg laying of experimental race multivoltine mulberry pure Mysore were kept in incubation at 26±1ºC till hatching, after hatching they were brushed with feathers and kept in a rearing tray and fed with tender leaf and chopped mulberry leaves. Silkworm larvae were reared up to third moult in conventional leaf feeding method. After the third moult, the larvae were divided into 2 batches and each batches with three replications. Each replication was maintained with 200 larvae in each rearing tray. One batch was treated as control (protected rearing), whereas experiment was conducted by taking all precautionary measures in the rearing room to prevent the entry of *Bacillus thuringiensis.* Another batch was considered as experimental (open rearing) where rearing was conducted under uncontrolled condition in a rearing room, where no precautionary measure was taken to prevent the entry of *Bacillus thuringiensis*, and rather than there had been the provision for *Bacillus thuringiensis* to oviposit. Oviposition of *Bacillus thuringiensis* on reared silkworm larvae and their subsequent development of sotto bacterial disease had been studied elaborately and recorded.

During the whole experiment the Oviposition behavior of *Bacillus thuringiensis* was marked meticulously. All the rearing data of the experiment as well as relative humidity 80±5%RH and temperature 26±1°C maintained in the rearing room were recorded during the experiment. Incidence of *Bacillus thuringiensis* and also the pattern of oviposition were recorded in every experiment along with control in the respective seasons. Pattern was recorded on the basis of position of the rearing tray placed in the rearing room serial wise from top to bottom. Study of *Bacillus thuringiensis* **(Vaughn, et. al, 2005)** infestation during rearing of mulberry silkworm in different meteorology in laboratory was undertaken to overcome the infestation of *Bacillus thuringiensis* in silkworm in context of open and protected rearing observation as detailed in tables, along with observation of weather parameters and effect of meteorology fluctuation.

Results and Discussion

Investigations from the study revealed that the infestation caused by *Bacillus thuringiensis* (Sotto disease) during rearing of mulberry silkworm, *Bombyx mori* L. is significantly lower (1.99%) in the fifth instar silkworm larvae and 1.21% in pupal stage in protected rearing of summer meteorology, compared to open rearing of summer meteorology **(Table 1)**.

Table: 1. Effect of *Bacillus thuringiensis* infestation sotto bacterial disease in summer and rainy meteorology.

Different stages of silkworm larvae	*Bacillus thuringiensis* infestation sotto bacterial disease			
	Summer meteorology		Rainy meteorology	
	Protected rearing of silkworm	Open rearing of silkworm	Protected rearing of silkworm	Open rearing of silkworm
3rd instar larva	00	00	00	00
4th instar larva	00	7.0	00	8.87
5th instar larva	1.99	9.88	3.88	14.57
Pupation stage of silk larva	1.21	7.66	3.05	15.05
Total infestation	3.20	25.0	7.99	40.64

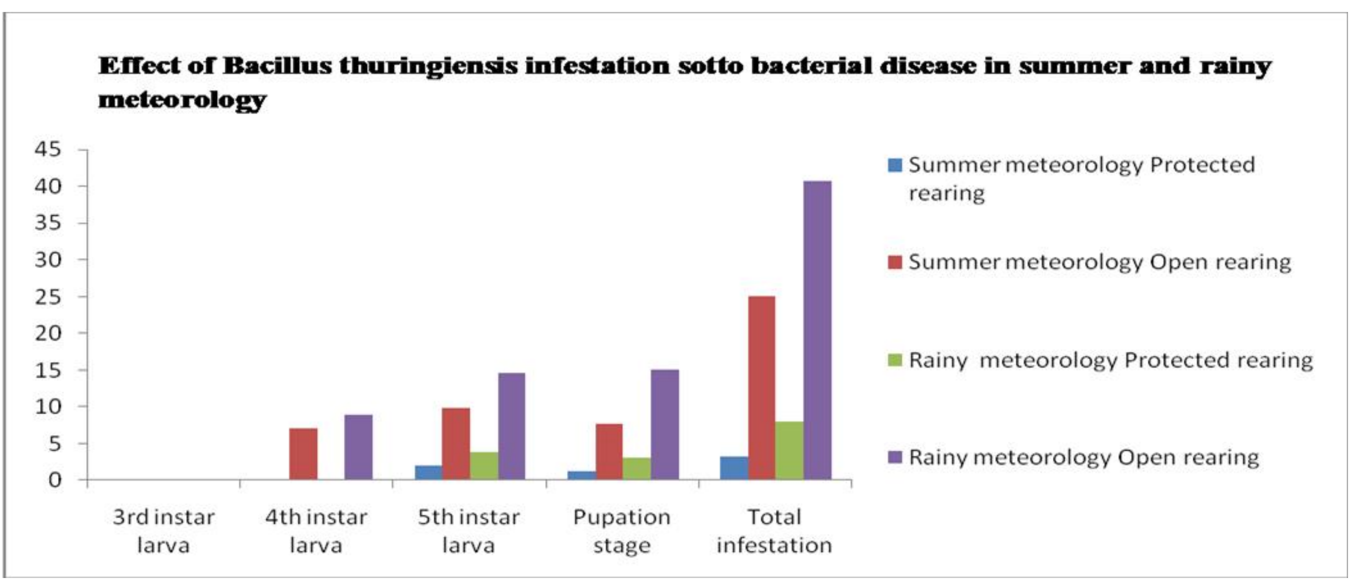
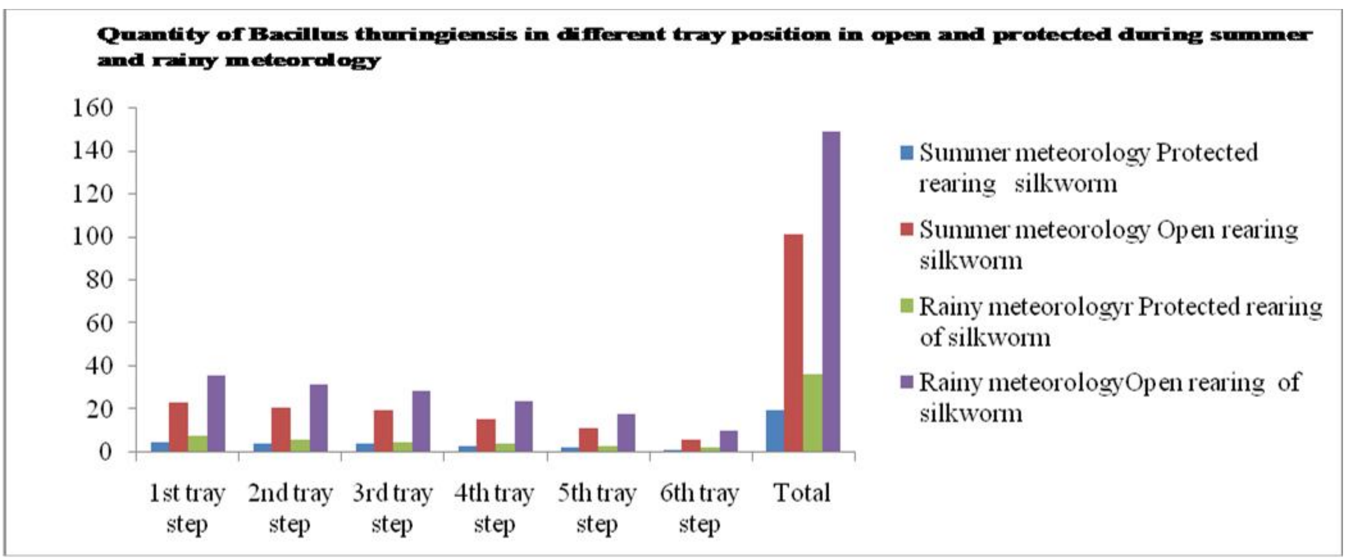


Table: 2. Quantity of *Bacillus thuringiensis* in different tray position in open and protected during summer and rainy meteorology.

Tray position Upper to Bottom	%Quantity of *Bacillus thuringiensis* in summer meteorology in different rearing conditions		%Quantity of *Bacillus thuringiensis* in rainy meteorology in different rearing conditions	
	Protected rearing Of silkworm	Open rearing of silkworm	Protected rearing of silkworm	Open rearing of silkworm
1st tray steps	4.50	23.00	7.81	35.58
2nd try steps	4.01	21.00	5.92	31.44
3rd try steps	3.88	19.50	4.80	28.52
4th try steps	3.02	15.50	3.87	23.88
5th try steps	2.42	11.50	3.11	17.77
6th try steps	1.45	5.90	2.23	10.12
Total	19.50	101.20	36.50	148.50



The data obtained from open rearing of summer meteorology revealed that *Bacillus thuringiensis* infestation started at the beginning of 4th instar affecting 7.0% of the silkworm larvae. *Bacillus thuringiensis* infestation at 5th instar was 9.88% and in pupal stage 7.66% infestation was recorded. In case of rainy meteorology, *Bacillus thuringiensis* infestation was significantly higher i.e., 3.88% in the 5th instar silkworm larvae and 3.05% infestation in pupal stage of protected rearing, compared to open rearing of rainy meteorology, whereas in the open rearing the *Bacillus thuringiensis* infestation started at the beginning of 4th instar affecting 8.87% of the larvae and in the 5th instar 14.57% infestation and in the pupal stage 15.05% infestation were observed. The *Bacillus thuringiensis* infestation was least in the summer meteorology protected rearing in 5th instar larvae (1.99%) as well as pupal stage (1.21%), whereas in open rearing the *Bacillus thuringiensis* infestation started at the beginning of 4th instar (7.0%) and in 5th instar (9.88%). These results indicated that a *Bacillus thuringiensis* infestation got higher in the rainy meteorology as compared to the summer meteorology. These results are in accordance with **(Narayanaswamy, 1991)**. According to him, incidence reached the maximum during the monsoon meteorology i.e., from June to September and least in summer meteorology (February to May). According to him the longevity of the *Bacillus thuringiensis* is reported to be inversely proportional to temperature i.e., higher the temperature lower the adult longevity. However, the tendencies of *Bacillus thuringiensis* oviposition more on the top steps trays **(Table 2)** as soon as it enters, instantly gets attracted to the top step trays and then moves downwards. The single gravid insect pest lays eggs not more than 2 to 3 per silkworm larvae, so that it can cover the maximum population. Incidence and infestation of *Bacillus thuringiensis* in cocoon stage in the tune 1.21% and 3.05% in protected rearing and 7.66% and 15.05% in open rearing in summer and rainy meteorology, respectively. It clearly indicated that in these cases oviposition time was during the spinning stage of the larvae only because the insect pest egg after hatching enter into the spinning larvae and metamorphosed into sotto diseased eggs within a week, by this time the silkworm larvae managed to spin the cocoon and pest eggs escaped from the cocoon by damaging the larva or pupa inside, through a small puncture by the release toxin to break development but the pupa of silkworm release cocoonase enzyme and thus metamorphosed into pupa outside the cocoon. In reality, the favorable environmental conditions are conducive for the exponential growth of *Bacillus thuringiensis* population as recorded 25.0% and 40.64% infestation, respectively in experimental batch in summer and rainy meteorology since temperature 26±5°C and 26±7 °C, respectively in summer and rainy and relative humidity 70±5%RH and 80±5%RH, respectively in summer and rainy were at optimum level for the growth and development in laboratory condition.

These results are in accordance with **(Sarkar et al., 2020)**. He reported that the rainy season is more prone to fly attack than summer Karnataka is the leading silk producing state of the country due to favorable climatic conditions for silkworm rearing as well as host plant production. Entomological bacteria are defined **(Vadlamudi, et. al. 1995; Shelton, et al. 2002 and Li, M. S. 2007)** by their production of pathogenic factors to cause disease in mulberry insect. Bt seems to be indigenous to diverse environments. Strains have been isolated worldwide from many habitats, including soil, insects, stored-product dust, and deciduous and coniferous leaves **(Bernhard, et. al. 1997; Chaufaux, et. al. 1997and Martin, P. A. 1989).** Bt belongs to the Bacillaceae family and is closely related to Bacillus cereus **(Jensen, G. B., 2003 and Lee, M., D.et. al. 2006).**The only notable phenotypic difference between the two species is the production of one or more insecticidal crystals. Although taxonomically diverse these bacteria commonly use the alimentary tract to enter the host and virulence factors target disruption of the gut epithelial barrier to reach the haemolymph and cause death by *Bacillus thuringiensis* sotto disease. Moreover, silkworm rearing is continuous throughout the year alternated with seed crop and commercial crop. It can be concluded from the experiment that protected rearing is more beneficial than open rearing where no precautionary measures are indicated to prevent *Bacillus thuringiensis*. Best silk yield can be obtained in the summer meteorology following the protected rearing practices.

Conclusion

This paper will discuss well characterized and more recent study of gram positive *Bacillus thuringiensis* bacterial entomopathogens. Aspects discuss include the basic meteorology, ecology and biology of each species, followed by detailed presentation of the sotto bacterial disease process and main pathogenic factors *Bacillus thuringiensis* involved. Main disease affecting pest during rainy meteorology but summer meteorology best yield of silk obtained. The study included future strategies to be taken for the management of meteorology and *Bacillus thuringiensis* pest condition for successful cocoon crop.

References

1. Bernhard, K., P. Jarrett, M. Meadows, J. Butt, D. J. Ellis, G. M. Roberts, S. Pauli, P. Rodgers, and H. D. Burges. (1997). Natural isolates of *Bacillus thuringiensis*: Worldwide distribution, characterization, and activity against insect pests. J. Invertebr. Pathol. 70: 59-68.
2. Bhattacharya S S, Chakraborty N, Sahakundu A K. (1993). Influence of abiotic factors on uzifly *Exorista sorbillans* (Widemann) Beck, infestation on silkworm (*Bombyx mori* L.) and assessment of crop loss in Malda district (West Bengal, India), Sericologia 33(2): 289-300.
3. Chaufaux, J., M. Marchal, N. Gilois, I. Jehanno, and C. Buisson. (1997). Investigation of natural strains of *Bacillus thuringiensis* in different biotopes throughout the world. Can. J. Microbiol. 43: 337-343.
4. Crickmore, N., D. R. Zeigler, E. Schnepf, J. Van Rie, D. Lereclus, J. Baum, A. Bravo, and D. H. Dean.(2006). *Bacillus thuringiensis* toxin nomenclature. https://www.lifesci.susex.
5. Dandin S B, Jayaswal J, Giridhar K. (Eds.) (2001). Silkworm diseases and pest and their control measure, Handbook of Sericulture Technologies, (2nd edition.) Central Silk Board, pp: 251-254.
6. De Maagd, R. A., A. Bravo, and N. Crickmore. (2001). How *Bacillus thuringiensis* has evolved specific toxins to colonize the insect world. Trends Genet. 17: 193-199.
7. Feitelson, J. S., J. Payne, and L. Kim. (1992). *Bacillus thuringiensis*: Insects and beyond. Bio/Technology 10: 271-275.
8. Hofte, H. and H. R. Whiteley. (1989). Insecticidal crystal proteins of *Bacillus thuringiensis*. Microbiol. Rev. 53: 242-255.
9. Jensen, G. B., B. M. Hansen, J. Eilenberg, and J. Mahillon. (2003). The hidden lifestyles of *Bacillus cereus* and relatives. Environ. Microbiol. 5: 631-640.
10. Krishna Rao J V. 2009. Seasonal Occurrence and Control of Silkworm Diseases, Grasserie, Flacherie and Muscardine and Insect Pest, Uzi fly in Andhra Pradesh, India. International Journal of Industrial Entomology 18(2):57-61.
11. Lee, M., D. Bae, D. Lee, K. Jang, D. Oh, and S. Ha. (2006). Reduction of *Bacillus cereus* in cooked rice treated with sanitizers and disinfectants. J. Microbiol. Biotechnol. 16: 639-642.
12. Li, M. S., J. Y. Choi, J. Y. Roh, H. J. Shim, J. N. Kang, Y. Kim, Y. Wang, Z. N. Yu, B. R. Jin, and Y. H. Je. (2007).Identification of molecular characterization of novel cry1-type toxin genes from Bacillus thuringiensisK1 isolated in Korea. J. Microbiol. Biotechnol.(In press.)
13. Marroquin, L. D., D. Elyassnia, J. S. Griffitts, J. S. Feitelson, and R. V. Aroian. (2000). *Bacillus thuringiensis* (Bt) toxin susceptibility and isolation of resistance mutants in the nematode *Caenorhabditis elegans*. Genetics 155: 1693-1699.
14. Martin, P. A. and R. S. Travers. (1989). Worldwide abundance and distribution of *Bacillus thuringiensis* isolates. Appl. Environ. Microbiol. 55: 2437-2442.
15. Narayanaswamy K C. (1991). Population dynamics of Indian uzifly, *Exorista bombycis* (Louis) (Diptera: Tachinidae). Ph.D. Thesis, UAS, Bangalore.: 223-224.
16. Sarkar K, Biswas M, Das P. (2020). Studies on infestation of uzifly during silkworm rearing in different seasons in West Bengal at laboratory condition. Journal of Entomology and Zoology Studies 8(4): 1740-1744.
17. Shelton, A. M., J. Z. Zhao, and R. T. Roush. (2002). Economic, ecological, food safety, and social consequences of the deployment of Bt transgenic plants. Annu. Rev. Entomol. 47: 845-881.

18. Vadlamudi, R. K., E. Weber, I. Ji, T. H. Ji, and L. A. Bulla 1 Jr. (1995). Cloning and expression of a receptor for an insecticidal toxin of *Bacillus thuringiensis*. J. Biol. Chem. 270: 5490-5494.
19. Vaughn, T., T. Cavato, G. Brar, T. Coombe, T. De Gooyer, S. Ford, M. Groth, A. Howe, S. Johnson, K. Kolacz, C. Pilcher, J. Purcell, C. Romano, L. English, and J. Pershing.(2005). A method of controlling corn rootworm feeding using a *Bacillus thuringiensis* protein expressed in transgenic maize. Crop Sci. 45: 931-938.
20. Zhong, C., D. J. Ellar, A. Bishop, C. Johnson, S. Lin, and E. R. Hart. (2000). Characterization of a *Bacillus thuringiensis* δ-endotoxin which is toxic to insects in three orders. J. Invertebr. Pathol. 76: 131-139.

Access this Article in Online	
	Website: www.ijarbs.com
Quick Response Code	Subject: Plant Pathology
DOI:10.22192/ijarbs.2023.10.12.004	

How to cite this article:
Sanjai Kumar Gupta, Vikas Singh, Ram Pravesh & Purshottam Yadav. (2023). Study of bacterial sotto disease (*Bacillus thuringiensis*) infestation during rearing of mulberry silkworm in different meteorology. Int. J. Adv. Res. Biol. Sci. 10(12): 30-36.
DOI: http://dx.doi.org/10.22192/ijarbs.2023.10.12.004