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EFFECT OF *BACILLUS CEREUS* INFECTION ON HAEMOCYTE COUNT OF THE MOLLUSC *TRACHIA VITTATA*

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ABSTRACT

The mollusc *Trachia vittata* infected with bacterium *Bacillus cereus* showed asignificant rise in the haemocyte count at first of post infective period in all dilutions when comparedwith non infected control. Thereafter from 3rd day onwards, declined to certain level comparedto previous day observation which is continued upto 15th day of experiment. But the decline of 3rd drops down markedly below to the control value at 10^{-6} and 10^{-5}. At 4th day of observation, thehaemocyte count of all dilutions showed a significant drop compared to the control.

Key words : Haemolymph, Haemocyte, Defence mechanism, *Trachia vittata*, *Bacillus cereus*.

INTRODUCTION

Molluscs have an open circulatory system and the circulatory fluid of molluscs ishaemolymph, which is slightly bluish in colour due to the presence of respiratory pigmenthaemocyanine. Haemolymph transports nutrients, respiratory gases, and enzymes, metabolicwastes and also toxicants throughout the body. Haemolymph with plasma and corpusclescan provide information pertinent to health assessment of animals or population. Haemocytesrepresent one of the most important defense mechanisms againstforeign materials in Mollusca. A large number of colourless stellate amoebocytes or corpusclesalso referred as leucocytes are found in plasma, which is collectively called as haemocytes(Gustafson & Stoskop 2005). Haemocytes of marine bivalve molluscs are believed to playa primary role in internal defense (Feng *et al* 1977, Cheng 1981) and responsible for woundplugging and phagocytosis or encapsulation of invading microorganisms, wound and shellrepair, transport and digestion of nutrients and internal defence (Cheng 1981, 1984, Fisher1986).

Molluscan defense mechanisms are regulated to innate immunity, which is largelydependent on cellular components such as haemocytes possessing phagocytic and bactericidalactivities. Among immune responses, apoptosis is an indispensable process because it enablesthe adequate clearance of damaged, senescent and infected cells without inflammation. Thenumber of haemocytes present in the heaemolymph depends on several factors such as age,temperature, infections (Noda & Loker 1989), parasite invasion (Drozdowski & Zbikowska 1994), injuries (Sminia 1981), water content in the tissues (Zbikowska 1998) and generalcondition of the organism (Barracco *et al* 1993). Older individuals may have a twice highernumber of blood cells than young animals. On the basis of morphology, two basic cell typesare recognized among molluscan haemocytes (Carballal *et al* 1997), Agranulocytes(hyalinocytes) and granulocytes (Adamowicz & Bolaczec 2003). The number of agranulocyteswas more in *Lamellidens marginalis* than *Biomphalaria bengalensis*, whereas number ofGranulocytes was more in *B. bengalensis* than *L. marginalis* which indicates that haemoraldefense mechanism was stronger in freshwater snail *B. bengalensis* as compared to bivalve*L. marginalis* (Kambale & Potdar 2010). Hence, in the present study, the number ofhaemocytes and the granulocytes of *Trachia vittata* infected by *Bacillus cereus*were analyzed and compared to non-infected snails to asses the role of haemocytes indefence mechanism.

MATERIALS AND METHODS

Collection of Haemolymph The mature control and bacterium*Bacillus Cereus* injected *Trachia vittata* culturing inlaboratory condition were kept on filter paper for 2 to 4 hours for the removal of water and mantle fluid.Shells were carefully opened apart to a width of 4 mm and the water present in the mantle was drainedout, and blotted dry with tissue paper. A hypodermic needle of 23G was inserted into the posterioradductor muscle and about 1.5 ml of haemolymph withdrawn. The entire operation was done withutmost care giving minimum disturbance to the animal. The collected haemolyph was poured into thesmall test tubes packed around with ice and diluted with physiological saline in 1:1 proportion. Followingthe same haemolyph collection procedure, the haemolyph was collected in all bacterial injected *T. vittata*at 1st, 3rd, 5th, 7th and 15th days of post infective periods.

Haemocyte counts : Total haemocyte counts (THC) were made using improved Neuber chamber. A one hundred µl sample of haemolymph was placed on a haemocytometer and haemocytes were counted out andexpressed as cells x 10^{-6} cells/ml haemolymph.

Preparation of physiological solution : The physiological saline was prepared in the following mineralscomposition and adjusted to pH 7.3.The snail *Trachia vittata* selected for the present study is injected with different dilution of

bacterium *Bacillus cereus*,. The changes in the number of haemocytes were counted in the haemolyph of pathogen injected *T. vittata* at 1st, 3rd, 5th, 7th and 15th day of infection to asses theirdefence mechanism.

RESULTS& DICUSSION

The percentage over control haemocyte count in all dilutionsuch as10^{-8},10^{-7}, 10^{-6} and10^{-5} was

Days	**Serial dilution**				
	Control	**10^{-8}**	**10^{-7}**	**10^{-6}**	**10^{-5}**

recoded as 25.14,12.63,3.22, -13.90 and -38.25 ; 81.01,41.21,12.36, -5.88, -21.19 and 32.24; 91.06,68.13,53.76,9.63,-10.33 and 28.42; 101.68,76.37,59.14, 40.64, -4.89 and 21.31 respectively at all experimental days such as 1^{st} 3^{rd} , 5^{th} 7^{th} and 15th when compared to their respective control.

1st Day	180±17.2	232±19.1	321±30.1	335±30.2	372±35.1
2nd Day	183±18	205±18.3	284±25.2	307±28.7	341±33.5
3rd Day	186±18.4	195±19.2	253±22.3	271±25.5	305±28.2
5th Day	188±18.5	176±17.5	205±20.4	246±22.4	289±27.6
7th Day	185±17.7	153±15.2	182±17.6	208±18.3	264±24.8
15th Day	182±17.1	137±12.6	156±15.7	185±16.4	222±20.23

n=5;X ±S.E

Table-1 Variations in the heamocytes count of different dilution of *Bacillus cereus* infected snails *Trachia vittata* .

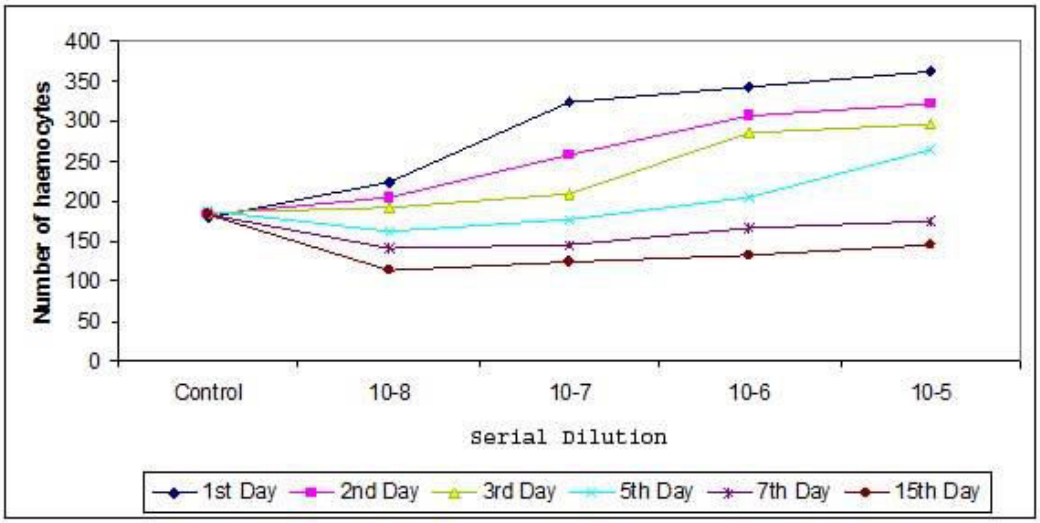

Figure- Variations in the heamocytes count of different dilution of *Bacillus cereus* infected snails *Trachia vittata*

Unlike vertebrates, molluscs have an ancestral defensive line and thisis comparable to that immune innate system in vertebrates and constituted by a cellular component and the products

that it generates (i.e. humoralcomponent). Haemocytes are thought to be involved in many functions, including digestion,metabolite transport and wound and shell repair (Sparks & Morado 1988). However, theirmost important role resides in internal defence (Cheng *et al* 1981). The circulating cells,called haemocytes are responsible for the phagocytosis, cytotoxic reactions, and the synthesisof the humoral factors, comprising antimicrobial peptides, agglutinins, lectins, cytokines, nitricoxide, etc; (Ottaviani 2006). Haemocytes are involved in various physiological functionsincluding nutrient digestion, transportation, and distribution and shell and tissue repair (Cheng1984; Beninger 2003; Sparks & Morado 1988). Haemocytes also mediate cellular internaldefence in molluscs through accumulation and detoxification of chemical toxicants (Fisher2004a b), phagocytosis and encapsulation (Karupa *et al* 1977; Chagot *et al* 1987, Montes*et al* 1997) of invading, foreign, biological material. By considering functional aspects,haemocytes can be distinguished as stem cells, phagocytes, trophic cells, haemostaticallyactive cells which are responsible for blood haemostasis, and (Glinski & Jarosz 1997). Ingeneral, a capsule of haemocytes encloses the foreign body and cytotoxic products, such asdegradative enzymes and free radicals, are released by the haemocytes in an attempt to destroy the invader. This results showedsome concurrence with the results observed by Cooper Willis (1979) who suggested thatduring the course of infection, a significant reduction occurred in the number of circulatinghaemocytes from 4 to 72 hours after exposure to *A. vasorum* L1 larvae ($P< 0.05$). Thisreduction was gradual through 5 hours after infection. From 6 to 72 hours after infection, thenumber of circulating haemocytes in the snails remained without much alteration, although ata significantly lower number than that in the control group ($P<0.05$). From10 to 60 daysafter infection, normal patterns of total haemocytes were reestablished in the haemolymphs,with no significant differences between groups ($P>0.05$) and these are further supported byMounkassa and Jourdane (1990) who found in the gastropod *Biomphalaria glabrata*challenged with bacteria, that the decrease in the number of circulating haemocytes was aresult of the mobilization of haemocytes in the tissues in response to an increasing demand for nutrients by the host and/or to repair wounds caused by the pathogen. Bezerra *et al*(1997) reported a reduction in the population of circulating haemocytes in *B. glabrata* in thefirst five hours after infection by *Schistosoma mansoni.* Studies carried out on *B. tenagophila*demonstrate that the temporary reduction in the number of circulating granulocytes results inincreased

susceptibility to infection by *Schistosoma mansoni* (Pereira *et al* 2006). In thecase of an infectious process, the capacity for haemocyte redistribution in the organismmight be interpreted as an increase in the efficency of the internal defense system by providingdefensively active cells at sites of pathogenic aggression (Smith & Ratcliffe 1980, Loker *etal* 1988).

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