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AN EFFICIENT INNATE IMMUNE RESPONSE OF *DANAUS GENUTIA* (LEPIDOPTERA: NYMPHALIDAE) LARVAE IS INDEPENDENT OF HEMOCYTE CONCENTRATION AGAINST *E. COLI*[1]

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The immunobiology of insects is different from that of vertebrates by having only nonspecific innate immune systems (Freitak et al., 2003). Many parasites and pathogens of bacteria, virus, fungi, protozoa and metazoa are known to infect insects during different developmental periods (Tunaz and Stanley, 2009). However, insects have their own strategies to combat the infection by cellular and humoral defense mechanisms of their nonspecific immune response (Gillespie, et al., 1997). The immune system of insects exhibits different components including hemocytes and an enzyme, phenoloxidase (Satterfield, et al., 2013). The hemocytes are the specific blood cells that play a central role in the immune response involving recognition, phagocytosis, and encapsulation of invading pathogens (Rolff and Reynolds, 2009; Satterfield et al., 2013).

Danaus genutia (Lepidoptera: Nymphalidae) is one of the common milkweed butterflies found in India. The butterfly is known as the milkweed butterfly since the larvae feed on milkweeds (Apocynaceae) which possess a high alkaloid content. Feeding on alkaloid-rich foliage makes the larvae unpalatable to their predators. Due to their abundance and attractive coloration, these butterflies are reared in both open and closed butterfly conservatories. However, it is estimated that the population of *D. genutia* has drastically declined in India during the past few decades (Atluri et al., 2010) due to various reasons.

The survival of any insect in its natural habitat is directly influenced by its immune response (Satterfield et al., 2013). In migratory butterflies, the energy allocation for movement has direct influence on the energy spent on immunity (Rolff and Siva-Jothy, 2003; Satterfield et al., 2013). However, the larval stages in butterflies are more susceptible than the adults to various microbial diseases. Infection decreases the lifespan, especially in migratory insects (Satterfield et al., 2013) like butterflies. There are several types of hemocytes present in the hemolymph which aid in resisting the physiological abnormalities induced by various pathogens. The present study was carried out to gather preliminary information

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regarding the hemocyte efficiency of *Danaus genutia* larvae against the common gram negative bacteria *E. coli*.

Eggs and larvae of *Danaus genutia* were collected from their host plants (*Asclepias curassavica* and *Cynanchum dalhousiee*) from Jnana Bharathi campus of Bangalore University and transported to the Insectary, Division of Biological Science. Larvae were fed with the leaves of both *Cynanchum dalhousiee* and *Asclepias curassavica*. Only third instar (and above) larvae were used for the experiments. *E. coli* cultures were obtained from the Department of Microbiology, Bangalore University and were maintained on nutrient agar. The cultures were kept in an incubator shaker (overnight) at 37°C. Subculturing was done with sufficient plates and broth after 24 hours of incubation. The hemolymph was collected from the larvae by severing the third proleg and then diluted with an anticoagulant solution (0.01 M ethylenediamine tetraacetic acid, 0.1 M glucose, 0.062 M NaCl, and 0.026 M citric acid, pH 4.6) and phosphate buffer (pH 7, 10 mM).

The hemolymph was then subjected to tenfold serial dilution in 10 vials containing 45µl distilled water. For each vial, 0.3 µl of *E. coli* was added and incubated overnight at 37°C in an incubator shaker. For each tube, optical density was measured at 600 nm through an Eppendorf biophotometer. The antibacterial activity of hemolymph was analyzed by the disc diffusion method. Three Agar plates were made up of 1ml *E. coli* suspension, each with four divisions which were a, b, c, d. A Watmann paper disc of 0.55mm dia. was used for the experiment with different volumes of hemolymph in each division. The zone of inhibition was recorded and analyzed in each division of all three plates.

The hemolymph was subjected to different dilutions to obtain different con-

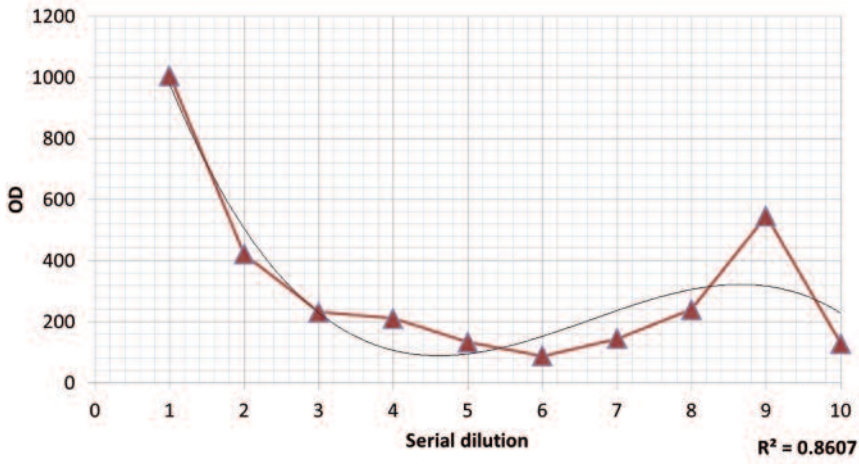
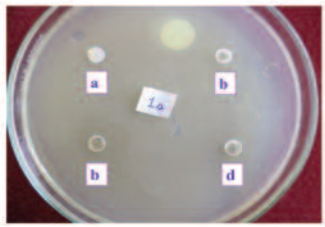


Figure 1: The variation in the bacterial proliferation observed to be independent of different concentration of hemolymph



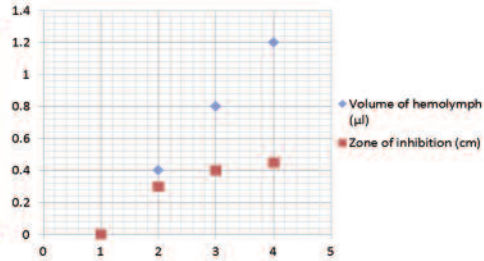
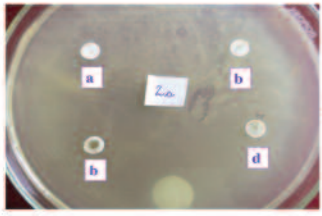


Plate No.	Division	Volume of E. coli (ml)	Volume of hemolymph (µl)	Watman paper width
1	A	1	DW	0.55
	B		0.4	
	C		0.8	
	D		1.2	



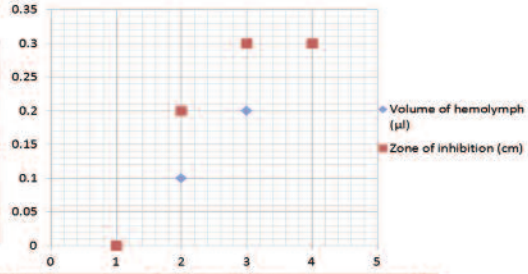
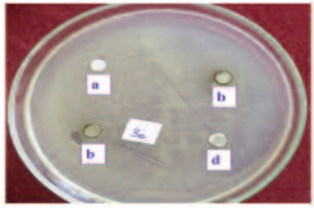


Plate No.	Division	Volume of E. coli (ml)	Volume of hemolymph (µl)	Watman paper width
2	A	1	DW	0.55
	B		0.1	
	C		0.2	
	D		0.3	



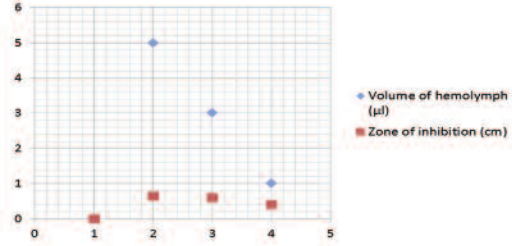


Plate No.	Division	Volume of *E. coli* (ml)	Volume of hemolymph (µl)	Watman paper width
3	A	1	DW	0.55
	B		5.0	
	C		3.0	
	D		1.0	

Figure 2: The disc diffusion method showing the zone of inhibition brought by different concentration of hemolymph has similarity in all the three plates

centrations against a constant bacterial load. The minimum concentration of hemolymph required for the inhibition of bacterial growth was determined by using the spectroscopic method and the resistance capacity was analyzed by the disc diffusion method. The higher concentration of hemolymph obviously could reduce bacterial growth but also the lowest concentration was good enough to inhibit the proliferation of bacteria (Fig. 1). Further, there was a steady variation in the bacterial proliferation which was not much influenced by hemolymph concentration (Fig. 2). The zone of inhibition was similar in all the divisions of the three plates.

Both these outcomes signify high efficiency of the larval immune system against *E. coli*. The hemocytes consist of several components which are essential for defense against various pathogens (Clark et al., 1997; Fang et al., 2010). The humoral immunity of the insect is equipped with antimicrobial peptides and the enzyme phenoloxidase, which critically eliminates the microbial infection from the insect system. Moreover, phagocytosis and encapsulation aids the cellular immune response along with the plasmatocytes (Clark et al., 1997). However, the present study did not specify the role of individual hemocytes against bacterial infection but instead focused on the overall performance of innate immunity. This preliminary information could be a good platform for further investigation in the immunobiology of this butterfly.

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