Impact of *Bacillus thuringiensis* on the hemolymph of *Locusta migratoria*

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ABSTRACT

This study shows the immune response of *Locusta migratoria* following treatment with 3 *Bacillus thuringiensis* strains. This study shows a reaction caused by the treatment with this bacterium. However, the reactions is identical for all 3 strains FAR4, HANA1, TIM24 that seem to cause the proliferation of plasmatocytes and granulocytes, unlike the prohemocytes seem to have a presence less marked than that of controls. This suggests that *Bacillus thuringiensis* causes an immune reaction

KEYWORDS: Hemolymphhe, Bacillus thuringiensis, Locusta migratoria, granulocytes, prohémocytes, plasmatocytes

INTRODUCTION

Several recent studies has been promising results to find products based on entomopathogenic bacteria that control the proliferation of pests, especially locusts that make up each year a real threat to humanity [2].

About 1,500 insect pathogenic microorganisms fungal, viral or bacterial origin are currently known. Among bacteria, only the *Bacillus* a genus of *Bacillaceae* has been used to combat insects; *Bacillus thuringiensis* is the specie that offers the most interesting potential insecticides both in current applications and in bacterial biopesticides development prospects [7].

Currently, the development of a *Bacillus thuringiensis* based biopesticide is a priority axis of research in the field of fight against predator’s crops. This bacterium has the ability during its sporation phase to produce a range of insecticidal toxins against several insects [6]. *Bacillus thuringiensis* is the most common means of control in the world. In the moment of being it represents more than 90% market biopesticides [1]. In the present work we studied the haemolymphatic reaction of *Locusta migratoria* treated with 3 strains of *Bacillus thuringiensis*

MATERIAL AND METHOD

Several strains of *Bacillus thuringiensis* FAR4, HANA1, TIM24 provided by the laboratory of Cellular and Molecular Biology, Bacteriology team, were used in the treatment of 19 adults of *Locusta migratoria* (10 spores / ml ). Five adults of *Locusta migratoria* were used as Controls
Insects were isolated in cages (30cm / 20cm). Controls were placed under conditions identical to those experienced.

Insects controls received 0.2 ml of distilled water while the treated insects undergo oral gavage using a syringe microliter, through which we injected 0.2ml oral bacterial solution $10^7$ spore / ml; the insect responds to the presence of liquid by a reflex swallowing and absorption. Badigeonnage of insects consists of covering the body of the locust with a brush dipped in the bacterial solution with the same concentration and the same dose. The leaves of grass (the food for treated locusts) were also sprayed with the same amount of bacterial solution and same concentration.

**blood smear Realization:**

According to the method of Guzo and Stoltz, [4], 0.2 ml of fresh hemolymph in our case, have been taken from adults of *Locusta migratoria* using a syringe microliter. We performed a puncture between the hind leg and thorax [8]. This volume was spread in a thin layer on a slide, the slide was air dried, then deposit on the droplets hémolyph, methylene blue, and a few drops of distilled water. The excess dye is then removed with distilled water by rinsing. GHEMSA is also added on the blade. Following this blade were rinsed with running tap water. After drying the air blade, we realized the mounting between slide and cover slip. The observation and examination of prepared slides were made using light microscopy (GX100 and GX10, GX1000).

**RESULTS AND DISCUSSION**

- Case *Locusta migratoria* witness: We noted the presence of a variety of cells: prohemocytes, plasmatocytes granulocytes and podocytes. However, the concentration of these cells was different. Indeed we noted a higher presence of prohemocytes followed by plasmatocytes and granulocytes, and finally podocytes.

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**Fig. 1:** Different sorts of hémocytes observed in *Locusta migratoria* control. A : Different types of hémocytes. Grx100. B : (PLS) : Plasmatocytes+, (GR) : Granulocyte++. Grx1000 C : Podocyte+.Grx1000 D : Prohémocytes +++. Grx400. +++ : High Density . ++ : Middle Density. + : Faible density.

- Case of *Locusta migratoria* treated with strains of *Bacillus thuringiensis* FAR4, HANA1 et Tim24 (Fig.2,3,4 and 5)
Fig. 2: Hemolymph cells of *Locusta migratoria* treated with FAR4. A: accumulation of hémocytes. Grx100. B: les granulocytes. Grx400.

Fig. 3: Hemolymph cells of *Locusta migratoria* treated with HANA1. A: granulocytes. Grx1000. B: accumulation of hémocytes. Grx100

Fig. 4: Plasmatocyte of *Locusta migratoria* treated with HANA1. Grx1000.

A blood smear was performed 48 hours after insect treatment by the bacterial strains of *Bacillus thuringiensis* at a dose of $10^7$ spores/ml; controls received 0.2 ml of distilled water. The results of this study are showed on figures 1, 2, 3, 4 and 5. Prohemocytes decreased in treated insects was noted, which could be explained by their multiplication and differentiation as they represent stem cells all other types of hemocytes. The same phenomenon was observed by Halouane [5] who tested Metharhizium anisopliae on *Shistocerca gregaria* and Adama Coulibaly. [3] have tested the effect of *Beauveria bassiana* and *Metharhizium flavoviride* on *Anacridium aegyptium*. Increased plasmatocytes and granulocytes were also noted in treated insects, which could be explained by their involvement in the organization of defense processes on the host following the introduction of the pathogen by phagocytosis, the plasmatocytes and granulocytes are the most active in this task as shown by the literature [9].

We have also seen a proliferation of hemocytes, accumulation and cell grouping in treated insects which induced some semblance coagulation of the hemolymph in *Locusta migratoria*, in contrast to what was observed by Halouane [5] who tested *Metharhizium anisopliae* on *Shistocerca gregaria* and Adama Coulibaly [3] have tested the effect of *Beauveria bassiana* and *Metharhizium flavoviride* on *Anacridium aegyptium* and noted a decrease in the concentration of hemocytes.

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