# Carbohydrate Pattern in Larvae of the Wax Moth, *Galleria mellonella* L. Infected with *Bacillus thuringiensis kurstaki*

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*(Received, June 30, 2005; Accepted August 8, 2005)*

## ABSTRACT

Four parameters of carbohydrates.*i.e,* total hydrolysable sugars (THS), total soluble sugars (TSS), reducing sugars (RS) , and non-reducing sugars (NRS), were estimated in both Dipel 2X (*Bacillus thuringiensis kurstaki*) treated by $LC_{50}$ (4.784 g/100 g diet) and untreated larvae of *Galleria mellonella*. The results showed a general decrease in percentage amounts of carbohydrates in the treated larvae. THS decreased to 2.2% in comparison with the control (3.08%) on the 1st day of the test and reached 2.8% and 3.1% on the 4th day in both larval groups, respectively. Also, the other carbohydrates demonstrated quantitative differences between treated and untreated larvae.

**Key Words**: *Bacillus thuringienssi kurstaki, Galleria mellonella*, infection, carbohydrates.

## INTRODUCTION

Carbohydrates are known to play an important role contributing to the structure and function of all insect tissues. They can be found in nuclei, cytoplasm, and cell membrane as well as in haemolymph and supporting tissues. Trehalose and glucose are the common sugars in the haemolymph of most insects (Wayatt and Kalf, 1957). Meanwhile, the concentration of trehalose in the haemolymph usually ranges between 8 and 60 mg/ml, depending upon the insect species, developmental stage, and sex. Glucose concentration is generally 10-fold lower than trehalose (Wyatt, 1967). Wigglesworth (1976) stated that in lepidopterous larvae, trehalose makes up over 90% of the blood sugar, while Sutcliffe (1963) found that the haemolymph trehalose of the silkworm, *Bombyx mori* larvae ranged between 6.6 to 15.9 mg/ml. Chippendale (1978) reported that in many insect species, the non-reducing disaccharide, trehalose (α-D-glucopyranosyl -α-D-glucopyranoside) is the predominant circulating saccharide that plays a central role in carbohydrate metabolism. Trehalose is an important reserve disaccharide because it is readily hydrolysed (by trehalase) to glucose, which is in turn oxidized to provide energy, its highest concentration is normally found in insect haemolymph.

Undetermined bacterial species that was injected into the haemocoel of *Manduca sexta* larvae induced a rapid drop in haemolymph glucose levels. The fall in glucose levels appeared to be primarily due to a temporary cessation of feeding by the infected larvae rather than an alteration in metabolism (Bedoyan *et al.*, 1992).

The present study follows up the percentage of total hydrolysiable sugars (THS), total soluble sugars (TSS), reducing sugars (RS), and non reducing sugars (NRS) levels in larvae of the greater wax moth, *Galleria mellonella* L. fed on diet treated with *Bacillus thuringiensis kurstaki,* and also in untreated larvae for five successive days post *B. t.* ingestion.

## MATERIALS AND METHODS

The stock culture of *G. mellonella* larvae was reared in the laboratory on the diet described by Ibrahim *et al.* (1984). Exactly, 1200 larvae of 3rd instar ($L_3$) were fed on diet treated with the $LC_{50}$ of *B.t. kurstaki* ( 4.784 g of the commercial *B. t.* product Dipel 2X/100g diet), thus half of them (ca. 600 larvae) could survive to be used in this study, beside 600 untreated larvae as control. Treated diet was introduced to larvae to fed on for 24hrs, after which they were transferred onto untreated diet. During the next 5 days post treatment, 100 larvae were picked daily and frozen as a group in liquid nitrogen to stop the physiopathological processes (Omar, 2004). The same was done by 100 larvae from the control. The samples were processed for determination of the total hydrolysiable sugars (THS), total soluble sugars (TSS), reducing sugars (RS), and non reducing sugar (NRS) in the whole body tissues.

**Total hydrolysable sugars (THS)**

A known weight (0.5g) of the ground larval samples was placed in a test tube then sulfuric acid (10 ml. 1N) was added. The tube was sealed and placed overnight in an oven at 100°C. The solution was then filtered into a measuring flask (100-ml) and completed to the mark with distilled water. The total hydrolysable carbohydrates were determined with the phenol-sulfuric acid method (Dubois *et al.*, 1956 and Khalil, 1981).

**Total soluble sugars(TSS)**

Total soluble sugars were determined in the ethanol extract using the phenol-sulfuric method according to Dubois *et al.* (1956) as described by Khalil (1981) as follows: One ml of aqueous sugars extract was mixed with phenol (1 ml/1.5% w/v) and concentrated sulfuric acid (5ml) was then added by fast delivery pipette. The mixture was then shaken gently and left to cool for 15 minutes. The blank sample was carried out using water instead of sugar solution. The absorbency of the developed yellow-orange color was measured at 490 nm. using pure glucose carried out a standard

curve.

**Reducing sugars(RS)**

Reducing sugars were determined in the ethanol extract, using dinitrosalicylic acid method according to Miller (1959).

**Non-reducing sugars (NRS)**

Non-reducing sugars were calculated by difference between the total soluble and the reducing sugars (Khalil, 1981).

## RESULTS AND DISCUSSION

The results of carbohydrate analyses that were determined during the 5 successive days of the test in both *B.t.kurstaki* treated and untreated *G. mellonella* larvae accounted as percentages of total hydrolysable sugars (THS), total soluble sugars (TSS), reducing sugars (RS), and non reducing sugars (NRS) are presented in Table (1).

**Total hydrolysable sugars (THS)**

Data presented in Table (1) show that the percentages of total hydrolysable sugars were higher in untreated larvae than in treated ones. However, TSH decreased from 3.08% in the control larvae to 2.2% in the treated ones 24 hrs after treatment, then decreased in both larval samples to the respective rates of 1.49 & 1.74; and 1.278 & 1.136% on the 2nd and 3rd day of the test, respectively. On the 4th day, their amount in treated larvae increased to 2.875% exceeding that level recorded at the 1st day post treatment. Similar increase was recorded also on the 4th day in the control larvae reaching 3.106%. THS level dropped clearly on the 5th day of the test to 1.505 and 1.91% in treated and control larvae, respectively.

**Total soluble sugars (TSS)**

As shown in Table (1), the level of TSS remained stable in the control larvae on the 1st and 2nd day of the test by the respective values of 0.493 and 0.491%. Meanwhile, this value increased in the treated larvae from 0.488% on the 1st day to 0.502% on the 2nd day after treatment. TSS levels recorded 0.547, 0.184 and 0.157% in the treated larvae compared to 0.390, 0.1815 and 0.177% in the untreated (control) larvae on the 3rd, 4th, and 5th day of the test, respectively.

**Reducing sugars (RS):**

This parameter of detected carbohydrates accounted the least amounts compared to the other estimated sugar groups (Table 1), but they were, in general, less in the *B. t.* -treated than in the untreated *G. mellonella* larvae(*i.e.,* quantitative differences).

RS levels reached 0.039 and 0.042; 0.363 and 0.385; 0.0152 and 0.0358; 0.0196 and 0.0236; and 0.0709 and 0.056% in the treated and control larvae on the five successive days of the test, respectively.

**Non-reducing sugars (NRS)**

Levels of NRS were nearly equal in both the *B.t.*-treated (0.449%) and untreated (0.451%) *G. mellonella* larvae on the 1st day post treatment, as shown in Table (1). On the next successive three days (2nd, 3rd and 4th days), NRS accounted levels in the treated larvae higher than those in untreated ones, being 0.139, 0.531 and 0.1644% for the treatment compared to 0.106, 0.3542, and 0.1579% for the control, respectively. Both levels dropped to respective rates of 0.0861 and 0.121 on the 5th day of the test, being higher in the control than in the treated larvae.

The reversed trend in levels of both RS and NRS in treated and untreated *G. mellonella* larvae on the 5th day of the test may be attributed to the applied dose of *B.t.kurstaki* as $LC_{50}$, which enable about 50% of the treated larval population to recover and survive. Thus, it could be the case on the 5th day of the test concerning certain physiological status as for RS and NRS. Trehalose is a NRS disaccharide (α-D-glucopyranosyl-α-D-glucopyranoside) predominant, circulating in insect body, and plays a central role in carbohydrate metabolism (Chippendale, 1978). Trehalose was recorded in insects' haemolymph by Wyatt and Kalf (1957), Sutcliffe (1963), Wyatt (1967), and Wigglesworth (1976). They mentioned that its highest level is normally found in insect haemolymph. Meanwhile, Bedoyan *et al.* (1992) recorded a rapid drop in haemolymph glucose levels in larvae of *M. sexta* ingested diet treated with undetermined bacterial species, but they stated that the fall in glucose levels appeared to be primarily due to a temporary cessation of feeding by infected larvae rather than an alteration in metabolism.

Table (1): Carbohydrates (%) in *B.t. kurstaki* treated (T) and control (C) larvae of *G. mellonella*.

| Days after treatment | THS | | TSS | | RS | | NRS | |
|---|---|---|---|---|---|---|---|---|
| | T | C | T | C | T | C | T | C |
| 1 | 2.200 | 3.080 | 0.488 | 0.493 | 0.039 | 0.042 | 0.449 | 0.451 |
| 2 | 1.490 | 1.740 | 0.502 | 0.491 | 0.363 | 0.385 | 0.139 | 0.106 |
| 3 | 1.278 | 1.136 | 0.547 | 0.390 | 0.0152 | 0.0358 | 0.531 | 0.3542 |
| 4 | 2.875 | 3.106 | 0.184 | 0.1815 | 0.0196 | 0.0236 | 0.1644 | 0.1579 |
| 5 | 1.505 | 1.910 | 0.157 | 0.177 | 0.0709 | 0.056 | 0.0861 | 0.121 |

THS= total hydrolysable sugars, TSS= total soluble sugars, RS= reducing sugars, NRS= non-reducing sugars.

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