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Haemolymph of *Tuta absoluta*: Collection procedure and chemical characteristics

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ABSTRACT

A technique used to collect haemolymph of the tomato leafminer; *Tuta absoluta* (Lepidoptera) fourth larval instar was described. It depends on centrifugation of the larvae at a given speed. This technique has the advantage that it enables collecting blood from *T. absoluta* larvae whereas manual expression of blood from the cut ends of prologs is impossible. Approximatry 11 µl of haemolymph from 8-10 larvae were collected after centrifugation for 10 min at 1500 r.p.m.. The chemical characteristics of haemolymph plasma collected were studied. The haemolymph of the tomato leafminer contains 29.27, 390, 79, 440, 62.5 and 137.5 µg / ml of chloride, phosphorus, calcium, magnesium, sodium and potassium, respectively. Na: k ratio was 0.45, indicating the presence of low sodium index as an advanced feature like other Lepidopterans. The measured high magnesium content, and alkaline pH (=7.3) might be due to *T. absoluta* food habit as a phytophagous insect. In general, the main characteristics of the tomato lefminer are more or less similar to that of other species of Lepidoptera. The centrifugation technique is an efficient method to collect blood from small insects, but the optimal velocity of centrifugation must be detected for each species.

Keywords: Haemolymph, collection, ions, Tomato leafminer, *Tuta. absoluta*

INTRODUCTION

The tomato leafminer (Meyrick) (Lepidoptera: Gelechiidae) is a devastating pest of tomato originating from south America. After its initial detection in eastern Spain in 2006, it rapidly invaded various other European countries and spread throughout the Mediterranean basin (Nicolas *et al.*, 2010).

Although much is known about the physical properties and chemical composition of insect haemolymph (Duchateau *et al.*, 1953; Florkin and Jeuniaux, 1974; Pelletier and Clark, 1995; Amin, 2008), only few papers reported for tomato leafminer which is considered as a relatively new pest in Egypt. This is might largely a result of the difficulty of collecting haemolymph from it, owing to its small size, and the tendency of haemolymph to clot within few seconds after puncture of the body wall preventing blood flow. Amin and Ellakwa (2013) studied the kinetic properties of phenoloxidases from the tomato leafminer.

The purpose of this paper, the first is to describe a technique used to collect haemolymph, and to present some of the chemical characteristics of *T. absoluta* haemolymph. This study, to the best of our knowledge, constitutes the first comprehensive analysis of the haemolymph plasma of *T. absoluta* in Egypt. This information might aid in the rational development of culture media for the *In vitro* tissue growth of the pest.

MATERIAL AND METHODS

The tomato leafminer larvae used in the present study were field collected from Benha city. They maintained in cages (75cm high, 60 cm deep and 50cm wide) on annual species of tomato leaves, at 25 °C with 16:8 L: D Photoperiod.

Collection of haemolymph:

During an investigation of biochemical changes in the blood of pesticides- poisoned insects, it became necessary to collect comparatively large samples of *T. absoluta* blood. For this purpose method was developed involving low speed centrifugation of the larvae which enables yielding the largest quantity of the haemolymph present in such small insect.

At first the fourth instar larvae were kept for 10 min at a refrigerator (4°C) to avoid their irritability when capturing by hand. To prevent contamination of the blood by the contents of the digestive tract, the mouth and anus must be sealed.

This is done by closing these openings with the aid of binocular by a commercial adhesive known as Amir purchased from market. This α – cyanoacrylated liquid adhesive is powerful and solidified around mouth and anus within 1-2 min at room temperature, to form an effective seal.

Following sealing of the body openings, the coxa of the first abdominal proleg was punctured, and the larvae placed upon a perforated eppendorf tube which inserted on the top of centrifuge tube. The eppendorf tubes were perforated by a neadle to make fine holes allow blood to flow through it to the centrifuge tube. One eppendorf can accommodate 8 to 10 larvae at a time.

The tubes are placed in a centrifuge and spun for 10 min. The blood is thrown down through the eppendorf as a clear plasma with the haemocytes massed as a sediment on the bottom of the tube. The cell- free plasma can be decanted off. The centrifuge setting for optimal yield of blood was 1500 r.p.m.. To prevent melanization and coagulation, the haemolymph was held at 4°C after collection and centrifugation was done in a refrigerator (4°C).

Chemical analysis:

A microflat tip electrode attached to Metrohm/ Brinkmann pH-102 model pH meter was used to measure pH. The haemolymph plasma (10µl) was placed on a teflon plate and the pH was measured directly.

Inorganic phosphate was determined as described by Rockstein and Herron (1951). Calcium was estimated by a method using calcium dye complexes as recommended by Bio-Analytica Co. (USA) kit. Magnesium was determined according to Zettner and Seligson (1963). Chloride was determined by the thiocynate method used by a kit of Quimica applicada Co. (Spain). All the above ions were determined colormetrically. The absorbance of all reactions was measured by spectronic 1201 UV/Visible spectrophotometer (Milton Roy., Co., USA). Sodium and potassium were measured by a radiometer flame photometer as described by Amin and El-Halafawy (2001/2002).

Samples for the ions analyses were prepared by diluting haemolymph plasma in deionized water at the ratio 1:10. Standard deviations of the means were calculated by the computer using Costat program.

RESULTS AND DISCUSSION

Withdrawing blood from small insects like *T. absoluta* larvae is a difficult process. Thus manual expression of blood from the cut ends of prologs of tomato leafminer larvae is impossible. For this reason, centrifugation technique for blood collection was developed.

Results (Table1) shows that the blood volume collected increased by increasing time of centrifugation at 1500 r.p.m. for 10 min.

The centrifugation for 20 min not significantly increased blood quantity collected indicating that 10 min of centrifugation is sufficient for obtaining the most amount of blood available in the haemoceol. By this method one could collect about 11 µl of haemolymph from 8-10 larvae per one eppendorf tube after centrifugation for 10 min.

The centrifuge setting for optimal yield of blood was 1500 r.p.m., giving a relative centrifugal force of 443x gravity. This a relatively low speed of centrifugation was chosen to avoid rupture of internal body structures. Sternburg and Corrigan (1959) reported that, blood can be collected from the American cockroaches within a range from 2000 -2300 r.p.m.

Table 1: Effect of centrifugation time at 1500 r.p.m. on quantity of collected blood from *Tuta absoluta* fourth larval instar.

Centrifugation time (min)	Collected blood volume (µl)
2	3.5 ± 0.2
5	7 ± 0.5
10	11 ± 0.7
20	12 ± 0.6

Dissection of *T. absoluta* larvae under the binocular showed no effect on the digestive system.

The relatively small amount of blood collected (approximately 1 µl) Per 1 larvae is due to small size of larvae, since the last larval instar i.e the fourth instar of *T. absoluta* measures 3.5-5 mm. In the mosquito; *Anopheles stephensi*, approximately 0.1µl was collected from each recently emerged mosquito after centrifugation (Mack *et al*., 1978).

The ionic composition and pH of the haemolymph plasma in the fourth larval instar of *T. absoluta* is illustrated in Table (2). The haemolymph of the tomato leafminer contains 29.27, 390, 79, 440, 62.5 and 137.5 µg/ml of chloride, phosphorus, calcium, magnesium, sodium and potassium, respectively. Mg^{++} had the highest percentage in the blood, while Cl^{-} had the lowest one (Fig.1). In descending order, the studied ions quantity could be arranged as follows: Mg^{++}, Po_4^{---}, K^{+}, Ca^{++}, Na^{+}, and Cl^{-}.

Table 2: Ion Concentration (µg/ml) and pH of the haemolymph plasma in the fourth larval instar of *Tuta absoluta*.

Ions	Means ± SD	No. of samples
Chloride	29.72 ±1.11	4
phosphorus	390± 8.22	4
calcium	79 ± 2.3	5
magnesium	440 ± 11.9	3
sodium	62.5 ± 1.14	6
potassium	137.5 ± 5	6
pH	7.3 ± 0.1	4

-The results pooled from 10 larvae in each sample and 3 determinations.

- Na : K = 0.45

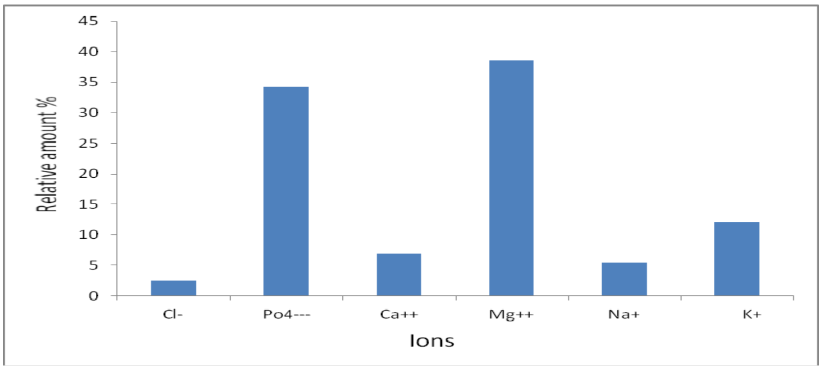

Fig. 1: The percentage of the anionic and cationic content of the haemolymph plasma of *Tuta absoluta* fourth larval instar.

Lepidopteran insects have low sodium index as an advanced or specialized feature (Duchateau *et al*., 1953). Na: K in the blood of tomato leafminer was 0.45. Amin and El-Halafawy (2001/2002) reported that K^+ ion level of the last three larval instars of the cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera) was very high as compared to that of Na^+ ion. Mostly, it exceeds 10 times more than Na^+. High magnesium content in the haemolymph plasma (= 440 µg/ml) found in *T. absoluta* is a characteristic feature of phytophagous insect (Pelleteir and Clark, 1995). Also, the slight alkalinity found in *T. absoluta* (pH= 7.3) might be due to their food habit as a phytophagous insect. In general, the main characteristics of the tomato leafminer are more or less similar to that of other lepidopteran insects.

The centrifugation technique is an efficient method to collect blood samples from small insects rather than collecting blood by manual expression of blood from legs or antennae, but it must be to take into consideration the optimal velocity of centrifugation for different insects to avoid blood contamination.

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ARABIC SUMMARY

طريقة جمع دم حشرة التوتا ابسيليوتا والخواص الكيميائية له

طارق رئيس أمين[1]- عزيزة حسن محمدي[2]

1- معهد بحوث وقاية النباتات ، مركز البحوث الزراعية ، الدقي- جيزة
2- المعمل المركزي للمبيدات ، مركز البحوث الزراعية ، الدقي- جيزة

أثبتت الدراسة أن جمع عينات الدم من الطور اليرقي الرابع لحشرة التوتا ابسيليوتا بجهاز الطرد المركزي عند سرعة معينة ، أنها وسيلة فعالة عن وسيلة استخراج الدم بالطريقة اليدوية نظرا لصغر حجم هذه الأفة. وقد تم ذلك عن طريق سد فتحتى الفم والشرج بواسطة مادة لاصقة لعدم تلوث الدم وعمل ثقب في مفصل أحد الأرجل للحلقة البطنية الأولى وتم وضع اليرقات في انبوبة مثقوبة والتي وضعت بدورها في انبوبة الطرد المركزي عند سرعة 1500 لفة / دقيقة لمدة 10 دقائق . وقد تم جمع كمية من الدم بواسطة هذه الطريقة تقدر بـ 11 ميكروليتر من 8-10 يرقات.

وعند تحليل الدم المتحصل عليه لدراسة بعض الصفات الكيميائية وجد أن أيون الأس الهيدروجيني يساوي 7.3 وأن الدم يحتوي على 29.27 ، 390، 79، 440، 62.5، 137.5 ميكروجرام/مللي من أيونات الكلورايد والفوسفور والكالسيوم والماغنسيوم والصوديوم والبوتاسيوم على التوالي. وقد تبين أن نسبة الصوديوم إلى البوتاسيوم أقل من الواحد الصحيح وتساوي 0.45 . وأن الدم غني بالماغنسيوم وهي الصفات المعروفة لرتبة حرشفية الأجنحة التى تتغذى على النباتات.