J. Hortl. Sci.
Vol. 15(2) 229-232 : 2020

Short Communication

First report on honeydew excretion by the melon thrips, *Thrips palmi* karny (Thysanoptera : Thripidae) and its biochemical analysis

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ABSTRACT

Sap sucking insects like thrips, aphids, mealybugs, whiteflies exploit the sugar rich phloem for growth and development. The excess sugar in the phloem sap creates osmotic imbalance leading to loss of water from haemolymph to gut lumen. In order to maintain osmolarity, sap sucking insects have developed structural adaptation (filter chamber) and also excrete excess sugar as honeydew through various orifices. The excreted honeydew is known to play very vital ecological role such as natural enemy calling (attracting parasitoids). In this regard scanty information is available on this important aspect for different sap sucking insects. In this study we are reporting for the first time on the composition of honeydew from the major horticultural thrips, *Thrips palmi* reared on French bean (*Phaseolus vulgaris*). LC-MS-MS analysis revealed the presence of 15 different sugars majorly inositol, fructose, maltose, glucose and sorbitol @ (130.9 ±0.47µg); (95.1±0.45µg); (60.7 ±0.28µg); (54.2 ±0.40µg) and (28.1 ±0.35µg), respectively.

Keywords: Honeydew, LC-MS-MS and Sugars and *Thrips palmi*

Sap sucking insects such as thrips, aphids, whitefly, mealybugs, leafhoppers, psyllids *etc*. feed primarily on the phloem sap which is rich in sugars such as sucrose, fructose, trehalose, maltose, raffinos, meteoritose *etc.*; free amino acids such as asparagine, glutamine, glutamate and serine (Hijaz and Killiny, 2014). Feeding of sugar rich sap leads to differential osmolarity between the hemolymph and gut lumen. To maintain the osmolarity between the gut lumen and the haemolymph, phloem feeders have developed several adaptations such as filter-chamber for efficient water usage and to excrete excess sugars in the form of honeydew through different orifices such as cornicles in aphids and anus in many other sap sucking insects. Honeydew acts as a medium through which insecticides are excreted and thereby contribute to the development of resistance to insecticides. This excretion of copious amount of honeydew on crops serves as substrate for the development of many saprophytic fungi like *Capnodium* sp., which affects photosynthesis (Lin, 2006; Wallace, 2008; Neto, 2011). It is also reported to be involved in natural enemy calling, which is self-inimical and provide food for ants which ensures dispersal and protection from the predators (Leroy *et al*., 2011). It has also been documented that honeydew is also a source of food for parasitoids involved in biological control. Rate of honeydew excretion and its composition by aphids and whiteflies is well studied, but the information on honeydew excretion by thrips is not studied in detail. Hence, a study was conducted to understand the pattern of honeydew excretion by the melon thrips, *T. palmi* due to its significance as a polyphagous pest and an important vector of *Watermelon bud necrosis virus* and *Groundnut bud necrosis virus* in India.

Stock culture of *T. palmi* was maintained on French bean (*Phaseolus vulgaris* CV. Arka Komal) pods at a temperature of 25±2°C and 68±% RH (Rebijith *et al*., 2011). One hundred adults of *T. palmi* were released on fresh French bean pods

This is part of Ph.D. thesis of first authour submitted to Jain (Deemed to be) University , Bengaluru

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of approximately equal size (3cm surface width; 13 cm length and 6 g in weight) which were harvested from the French bean plants grown under insect proof cages. The samples with thrips were placed inside a plastic container (10cm x10cm) and kept at the room temperature (25±2°C, 68±% RH). There were three replicates and same replicates of control (without inoculating *T. palmi*) were also maintained. Observations were made continually on the behavior of *T. palmi* adults and for the excretion of honeydew under the stereo-zoom microscope Stemi 305 (ZEISS, Germany).

Sugars were separated by following modified Steppuhn and Wackers (2004) method. After 24 h observation, the bean pods were washed with 10 mL of 80% ethanol, and the extract was evaporated and re-dissolved in mobile phase containing solvent A and solvent B in 1:1 ratio, filtered and injected to LC–MS/MS for sugar profiling.

Sugar standards *viz*. fructose, sucrose, galactose, glucose, maltose, fucose, rhamnose, xylose, arabinose, mannose, sorbitol, inositol, lactose, ribose and trehalose were purchased from Sigma Chemical Co., USA and calibration curve was prepared using different concentration of individual sugars. The mobile phase used was composed of solvent (A) 80:20 (Acetonitrile: Water) and solvent (B) 30:70 Acetonitrile: water with 0.1% ammonium hydroxide was filtrated through 0.2 µm nylon filter paper and separation was done using gradient elution. The initial gradient was composed of 100% solvent A for one min and at 8th min it was champed to 88% of solution A and 12% of solution B, which was held for 1 mint and a linear gradient was followed by 98% of solution A and 2% of solution B and at 15th mins it was held for 30 sec. The system had returned to initial settings at 19th min and equilibrated for 6 min. before the next injection and the flow rate was 0.1mL/min. The analytical column used was 2.1x10 mm UPLC BEH-Amide (Waters, USA) with 1.7 µm particle size and protected by vanguard BEH-Amide with particle size 1.7 µm. The column was maintained temperature of 25°C.

Study was conducted to understand the pattern of honeydew excretion by the melon thrips. Close observation under the stereo microscope revealed that *T. palmi* adults excrete honeydew which lasted for about 10 sec from initiation bending of abdomen to the release. These events were recorded in VLC format. Analysis of sugars in the honeydew revealed that there was a significant difference between the control samples and samples inoculated with thrips. Among the sugars estimated, inositol was the predominant sugar (130.95 ±0.47 µg/pod) in the honeydew followed by fructose (95.13 ±0.45 µg/pod); maltose (60.700 ±0.28µg/pod); glucose (54.22 ±0.40µg/pod); sorbitol (28.15 ±0.35g/pod) (Fig: 2) followed by less of lactose, mannose, galactose, arabinose, ribose and fucose (Table 1). It clearly indicated that the honeydew excreted by thrips is rich in soluble sugars. There is a single report on honeydew excretion by the red banded thrips, *Selenothrips rubrocinctus* (Giard) (Buss *et. al.,* 2006). Wool *et al*. (2006) reported about 20 sugars in the honeydew excreted by aphids. Glucose and fructose are basic components of the honeydew of sap feeding insects (Fischer *et al.,* 2005; Wool *et al.,* 2006). These sugars are present in honeydew in different proportions depending on the insect species and host plants. Hendrix *et al*. (1992) also observed the differences in sugar composition of honeydew excreted by *Trialeurodes vaporariorum* (Westwood) and *Bemisia tabaci* (Gennadius) feeding on different host plants.

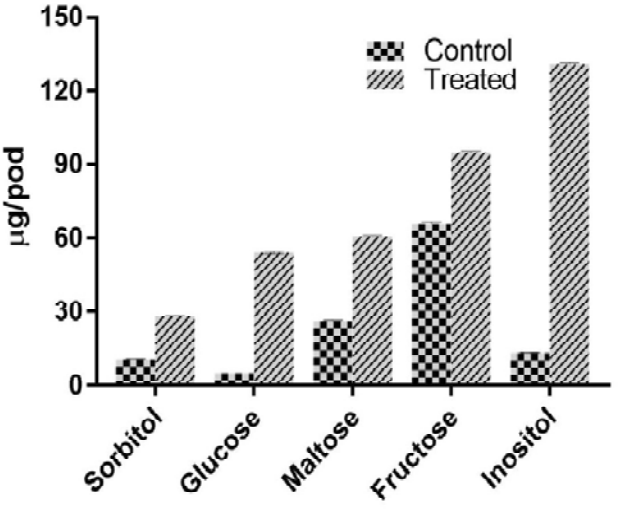

Fig. 1. Contents of major water soluble sugars in beans with or without thrips infestation

Table 1. Biochemical analyses of water-soluble sugars by LC-MS-MS in the bean pods with or without thrips infestation

Sugar	Control (±SE)	Treated (±SE)
Ribose	0.930±0.00	3.860±0.05
Arabinose	0.553±0.00	3.907±0.04
Xylose	3.690±0.08	0.683±0.02
Rhamnose	0.030±0.00	0.163±0.00
Fucose	0.010±0.00	0.020±0.00
Glucose	5.190±0.02	54.227±0.40
Fructose	66.253±0.56	95.137±0.45
Galactose	1.757±0.08	4.293±0.23
Mannose	5.100±0.09	5.253±0.07
Inositol	13.360±0.03	130.957±0.47
Sorbitol	10.660±0.09	28.153±0.35
Maltose	26.420±0.12	60.700±0.28
Lactose	0.907±0.00	3.820±0.04
Sucrose	0.343±0.00	0.890±0.01
Trehalose	0.010±0.00	0.030±0.00
Total	135.213±1.12	392.093±2.46

Differences in chemical composition of honeydew secreted by aphids are explained inter alia by genetic variation between insect populations (Fischer and Shingleton, 2001). However, the host plant sap is a primary factor that influence the diversity in biochemical composition of honeydew. Honeydew composition is an important factor in tri-tropic interaction involving natural enemies and also mediating ant–homopteran mutualisms. However, further studies on sugar composition in relation to species of ants attracted and its impact on predation/parasitism is required in order to have sustainable management of this pest.

ACKNOWLEDGEMENT

The authorsare thankful to the Director, ICAR-IIHR, Bengaluru for support and infrastructure facilities

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(Received on 27.02.2020 and Accepted on 27.12.2020)