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DISTRIBUTION OF SEROTONIN (5-HT) AND DOPAMINE (DA) ON DIGESTIVE TRACT OF RED PALM WEEVIL LARVA, *RHYNCHOPHORUS FERRUGINEUS* (COLEOPTERA: DRYOPHTHORIDAE)

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ABSTRACT

The red palm weevil (RPW), *Rhynchophorus ferrugineus* (Coleoptera: Dryopthoridae) is a well-known deadly pest of palmae tree worldwide. The larvae of RPW is the most destructive stage of its life cycle and caused great economic lost to palm tree plantation for example the coconut tree plantations. In this study, we focused on detection of two important biogenic amines, serotonin (5-HT) and dopamine (DA) that play important roles in the regulation of the digestive tract of this insect. Immunohistochemistry analysis has been carried out on the cross-sectioned tissue of three different parts of the digestive tract; foregut, midgut and hindgut. 5-HT receptor for serotonin and D1 receptor for dopamine were chosen for chromogenic

detection of the biogenic amine. The distribution of 5-HT can be detected at the plasma membrane, basement membrane and cytoplasm of the cell for the whole guts except at the midgut cell. While DA can only be detected at the basal membrane of the gut cell for the most part of the gut. Further study could be done to find a possible solution in controlling the survival of RPW by understanding the roles of the biogenic amine in controlling and modulating the mechanism of absorption and digestion of the digestive tract.

Keywords: serotonin, dopamine, red palm weevil, digestive tract

ABSTRAK

Kumbang Merah Palma (RPW), *Rhynchophorus ferrugineus* (Coleoptera: Dryophthoridae) merupakan serangga perosak utama kepadatan aman palma yang terkenal di seluruh dunia. Larva RPW merupakan peringkat perkembangan yang bertanggung jawab mengakibatkan kerosakan yang teruk padat tanaman palma dan menyebabkan kerugian yang besar pada tanaman seperti tanaman kelapa. Dalam kajian ini, kami memfokuskan untuk mengesan kehadiran dua jenis amina biogeni iaitu serotonin (5-HT) dan dopamin (DA) yang berkemungkinan memainkan peranan penting dalam regulasi sistem penghadaman larva RPW. Pengesanan melalui ujian imunohistokimia telah dilakukan pada keratin rentas tisu tiga bahagian usus salur penghadaman larva iaitu usus depan, usus tengah dan usus belakang. Reseptor umum untuk 5-HT serta reseptor D1 untuk DA telah digunakan dalam kajian ini untuk tujuan pengesanan melalui pewarnaan kromogenik. 5-HT telah Berjaya dikesan pada bahagian membran plasma sel, membrane dasar dan juga sitoplasma sel pada kesemua bahagian usus kecuali pada usus tengah. Manakala, DA hanya menunjukkan pewarnaan positif pada bahagian membrane dasar sel pada semua bahagian usus salur penghadaman larva RPW. Kajian

yang lebih lanjut perlu dijalankan untuk memahami dengan lebih lanjut dan mendalam mengenai peranan amina biogeni dalam pengawalan dan modulasi mekanisme pencernaan dan penyerapan pada salur penghadaman larva RPW.

Kata kunci: serotonin, dopamin, kumbang merah palma, salur penghadaman

INTRODUCTION

Red palm weevil (RPW) have cause a major lost in palm plantation as they are reported as a major pest for more than 26 palmae species. Originated from the Southeast Asia and Melanesia, it is now have been reported to infest the Middle East countries, Europe, Africa and the Northern America (EPPO 2015). In Malaysia it has caused a great damage to our coconut plantation as they were first detected at the coastal line of Terengganu in early of 2007 (Azmi et al 2013, Idris et al 2014). The concern of having the possibility of RPW infesting our palm oil plantation have caught our interest to understand more on its physiological function in a way to come out with a better solution.

The most destructive stage of this insect toward the palm tree are the larval stage. The larvae feed on the trunk of the palm tree thus creating empty cavities inside the tree that lead to the dead of the tree without any physical symptom appeared on the infested tree until the last stage of infestation. Although RPW is an economic important pest there are still no study conducted to understand the digestion system of this insect and the mechanism in supplying nutrition for their survival. The purpose of this study is to provide a fundamental information on the distribution of selected biogenic amine in order to

understand the mechanism of controlling and modulating the secretion and absorption of food in RPW larvae.

Digestive tract is the most important organs for insect for supplying the nutrient that needed for the growth and to carry out daily activities. The alimentary system of insect can be divided into three part: the foregut, midgut and hindgut (Wigglesworth 1982, Atkins 1978), however, different structure can be observed among species due to the differences in dietary habits as for the larva of RPW it has a large foregut structure compared to the other part of the guts as it is used to store large number of food. Meanwhile the midgut were the shortest region that consist of ventriculus and ileum, while the hindgut were coiled at the posterior part of the larva body (Harris et al 2015). We test the present of selected biogenic amine at all parts of the gut.

Biogenic amine plays a major role in regulation and mechanism of the behaviour and physiologically function (Falibene et al 2012). Serotonin (5-HT) and dopamine (DA) are classified in monoamine and catecholamine group. Of the three members of the catecholamine family (dopamine, noradrenaline, adrenaline), dopamine is the major amine found in insects (Evans 1980). Serotonin from the monoamines groups can act as neuromodulator, neurohormones or neurotransmitter, expressing their affects at the central and peripheral level (Orchard 2006, Scheiner et al 2006). Although both of them have a lots of specific receptor but we specifically test for D_1-like group for dopamine as it is a most likely receptor to be found on all important organ in human (brain, gastro-intestine) and 5-HT diverse range targeting receptor. We only stated the distribution of the biogenic amine at the digestive tract in this study while the function of this biogenic amine will not be discussed in details.

MATERIALS AND METHOD

Insects Sampling

Insect samples were collected from Kuala Terengganu, Terengganu, Malaysia by using combination of pheromone trap and sugar cane as a bait trap that were hang under the coconut tree, 1 meter from the ground. The trap were kept at a distance of 300 meter from each other.

Immunohistochemistry

Three parts of digestive tract were dissected and separated and fixed in 10% buffered formalin for 24 hours. The tissue were then washed through a series of ascending ethanol (70%, 80%, 90%, 100%, 100%) followed by sub-xylene. Tissue were embedded in paraffin wax and sectioned (3-4 µm) using a Leica microtome. The tissue slide undergo rehydration and mounted with DPX mounting medium.

Immunohistochemistry (IHC) staining started with dewaxing and rehydration process of tissue slide. Tissue slides were treated with Tris-EDTA pre-treatment solution (pH 8) for 30 minutes at 95 ºC. After cooling, the tissue slide is immersed in hydrogen peroxide (H_2O_2) solution for 6 minutes then the tissue section were rinsed with PBT (PBS + 0.2% bovine serum albumin + 0.1% TritonX-100) treated with primary antibody for 30 minutes. After rinsed with PBT the tissues were incubated with secondary antibody, horse-radish peroxidase (HRP) for 30 minutes and DAB Chromogen for 7 minutes. The tissues were rinsed with PBT and counterstain with Harris haematoxylin for 7 minutes. The slide undergo dehydration process with alcohol series and sub-xylene solution. Tissue sections were examined by Zeiss Axio Scope light microscope with iSolutionLite software.

RESULT AND DISCUSSION

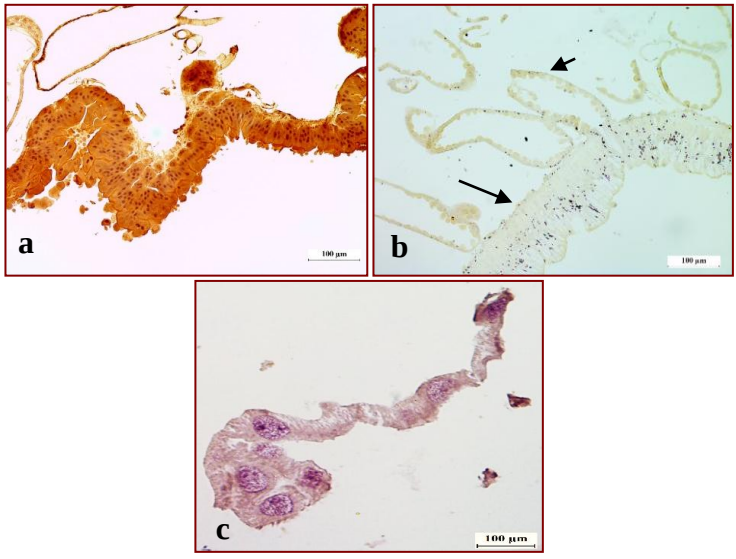

Figure 1 Detection and distribution of serotonin at the (a) Foregut (b) Midgut (c) Hindgut.

In this study, immunohistochemistry analysis of the RPW larvae gut revealed the presence of dopamine and serotonin on each part of the gut. Detections were made on specific receptors for each biogenic amine, 5-HT receptor for serotonin and D_1 receptor for dopamine. The result shows a positive chromogenic staining of DAB on the tissue sections that are test for serotonin on every part of the gut: foregut, midgut and hindgut but the distribution of the biogenic amine were different in each part. For the foregut, serotonin was detected at all parts of the cell except for the epithelium cell nucleus that shows blue staining from haematoxylin staining (Figure 1 (a)) For midgut and hindgut, the distributions of serotonin were concentrated at the basal membrane of the cell (Figure 1 (b)). However, there is some brownish coloured of the chromogen are stained inside the

cytoplasm of the rectum showing the same observation as the foregut (Figure 1 (c)). Other structures like the criptae papillae and Malpighian tubule of the larva gut shows a positive chromogenic staining with the same observation as the foregut.

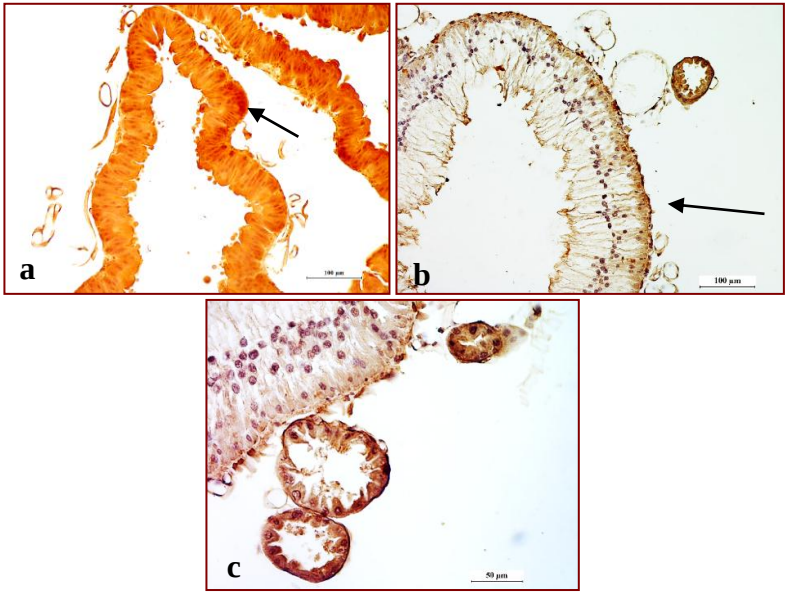

Figure 2 Detection and distribution of dopamine at the (a) Foregut (b) Midgut (c) Hindgut.

Chromogenic detection by IHC indicate the presence of dopamine at all three part of the gut; foregut, midgut, and hindgut by testing on the D_1 receptor site of dopamine. Meanwhile the distributions of dopamine were different for each part of the gut. As in the Figure 2 (a), dopamine was detected at all part of the cell including the nucleus showing an equal distribution but some were concentrated at the basement membrane of the epithelium cell. The circular muscles surrounding the cell were also showing the positive present of dopamine. Different observation of the distribution of dopamine

at the midgut where the chromogenic staining only present at the membrane of the epitelium cell and concentrated at the basement membrane. The cytoplasm and the nucleus were both seen to be translucent and the nucleus were stained by blue haematoxylin colour (Figure 2 (b)). The membrane of cell including the apical site of the cell is positive with brown colour of DAB chromogen staining. As for Malpighian tubule that are a part of the hindgut, the IHC test have confirmed that the dopamine were released at the hindgut cell. The dopamine has been detected at all part of the cell except for the nucleus of cell (Figure 2 (c)). The brown staining of the DAB chromogen was seen to be concentrated more at the basement membrane of the cell.

Dopamine and serotonin were both innervated in the mechanism and regulation of digestive tract of RPW larva as they were both positively distributed on all parts of the gut from foregut, midgut and the hindgut. Even though the real action of the serotonergic and dopaminergic effect on the secretion and absorption mechanism on the cell of the gut still need a further research, but as far as we know 5-HT, DA and other biogenic amine such as octapamine are involve as a neuromodulator affecting both pre- and post-synaptic site of motor neuron that keeping the mechanism of contraction, dilation and relaxation of gut muscle (Chapman 1998, Ayali 2004). It may act the same with this insect since both 5-HT and DA were widely distributed at the muscle layer at all part of the gut. This muscle action act as pump contraction that help the intake of food and keeping the flow of it inside the gut from part to another part of insect gut. As for example a test on ant that treated with 5-HT have increase the intake rate of food by increasing the pumping frequency on the first three hours but it shows a decrease on volume of solution taken per pump and a clear and significant depression were shown after further exposed with 5-HT

(Falibene et al. 2012). 5-HT also promote a decrease intake of sucrose, protein and carbohydrate based food in some insect such as plant-sap feeding aphids (Kaufmann et al 2004), cockroach *R. medera* (Cohen 2001) and flies (Dacks et. al. 2003). DA mechanism in controlling and modulating the gut are less to be studied.

Effect of 5-HT and DA as a neurotransmitter at the salivary gland of the RPW was denied by Hidayah (2013), that stated the mechanism of saliva secretion of their tubular gland were under hormonal control of 5-HT and DA since there were no innervation at the nerve fibre of the salivary gland. However in this study the immunohistochemistry test were only conducted on the cross section of the RPW larvae gut tissues, so we could not identified the innervation of this biogenic amine at the gut nerve fibres as it needs more work to test on the whole organ for immunohistochemistry or by using fluorescence detection. 5-HT and DA are not yet to be confirmed to either act as neurohormones or neurotransmitter in the regulation of the RPW larva digestive tract but it is clear to act as neuromodulator at the muscle layer surrounding the gut. Since they were both appeared to play an important role in the RPW larva gut, further experimental study need to be conducted as a part of this research to understand their effect on the modulation and control in RPW larvae gut.

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