Salivary gland (SG) is the most significant organ in insect feeding. Somehow, only few studies have been conducted to understand the salivary mechanism of Malaysian’s insects. Most work on salivary gland has been done overseas on pest species, for examples, the cockroach (*Periplaneta americana*) e.g. Just and Walz (1994), the desert locust (*Schistocerca gregaria*) e.g. Kendall (1969), and the plague locust (*Locusta migratoria*) e.g. Ali and Orchard (1996). Serotonin (5HT) and dopamine (DA) are two biogenic amines that were involved in many physiological events in several basic biological processes including the innervations in salivary gland.
Both amines shown to be potent neurotransmitters, neurohormones and neuromodulators in insect nervous system and various peripheral organs (Roeder 1994, Baumann et al. 2003). Serotonin and dopamine has been reported numerously to be the most important amines involved in the salivary gland innervations especially in cockroach, *Periplaneta americana* (Just and Walz 1996; Lang and Walz 1999; Baumann et al. 2004; Marg et al. 2004; Walz et al. 2006) and blood-feeding bugs, *Rhodnius prolixus* (Cook and Orchard 1990; Miggiani et al. 1999; Orchard and Brugge 2002; Orchard 2006).

Previous studies on the regulation in the salivary glands of cockroach indicated that salivation is control from these amines via dual innervation from subesophageal ganglion and stomatogastric nervous system (House, 1980; House and Ginsborg, 1985; Ali 1997a & 1997b; Zimmermann and Walz, 2003; Walz 2006). Watanabe & Mizunami (2006) reported that the salivary gland is regulated by the salivary duct nerve (SND) of two neurons with large axons (salivary neuron 1 (SN1) and salivary neuron 2 (SN2)) from its classical conditional study of salivary neurons activities in cockroach. SN1 in cockroach has been demonstrated to contains dopamine (Elia et al. 1994; Baumann et al. 2002) while SN2 neurotransmitter is still under investigation. Meanwhile, in locust, *Locusta migratoria*, the salivary secretions are also innervated by two pairs of neurons: SNI, contains dopamine, and the SN2, contains serotonin (Ali 1997a; 1997b; Ali and Orchard 1995). For blood-sucking insect, *Rhodnius prolixus*, the SG are innervated via the suboesophageal and stomatogastric nerves that are covered with a plexus of serotonergic immunoreactive fibres (Ali 1997b). If serotonin and dopamine involved in the salivary gland control of other insects, it is interesting to investigate the distributions these biogenic amines in the red palm weevil (RPW), *Rhynchophorus ferrugineus* (Olivier, 1790), to see the roles of these amines in the salivary glands regulations of the potential pest for our oil palm plantation.

Studies on RPW were actively carried out by entomologists from Middle East countries, Europe, India, China and Japan due to severe infestation by the red palm weevil (Abe et. al., 2009; Dutta et al., 2010; Li et al., 2009; Murphy et al., 2009; Roda et al., 2011). However, in Malaysia, very little published information
regarding this pest is available probably due there has been no serious infestation until recently in the state of Terengganu where almost all infestation observed on coconut trees (Sazali, Dept. Agric., 2012 – personal communication). Palm weevil is a very destructive insect pest to plants under the family of Palmaeae and also cacao, sugarcane, papaya, banana etc. in the tropics as well as in the southern part of the United States (Wattanapongsiri, 1966). This pest has cost billions of dollars lost in palm base industry such as dates palm in Arabic countries as well as coconut palm in India, ornamental palms in European countries and China (Abozuhairah, et al. 1966; Sekhar, 2000; Faleiro, 2006; Ju et al., 2006). The visible presence of symptoms of infection is unnoticeable until late infection (Murphy & Briscoe, 1999). During this stage it is already too late to control the pest because the palms had totally damaged and cannot be recovered. In recent years, this costly pest has been severely infesting East Malaysia attacking coconut trees and directly affecting the yield of the crops (Basari et al. 2011). Invasion of RPW if not control will soon reached out to palm oil estate and detriment to our oil palm industry. Prevention step has to be manifested to avoid outbreak of RPW population in oil palm estate. Further ignorant of this matter will only causing disaster to the oil palm industry. Bernama (2010) reported that the oil palm industry is one of the main industries in Malaysia that contributes RM 62.5 billion to Malaysia’s economy.In 2013, it may reach to RM 75 billion though CPO production seem to be negatively affected by the decline in efficiency of the Elaeidobius kamerunicus Faust, the main pollinator of oil palm flower (Ruslan Abdullah – Simedarby, personal communication)

Most of the study done so far only covers external morphology of RPW. To our knowledge, we are the pioneer in Malaysia to use a target study approach to understand the feeding system of the two variants of RPW, stripe and spotty, that have been suggested to be in one species when morphological characters, RAPD banding pattern, mitochondrial DNA sequencing, host plant preferences and pheromones production and response are alike based on a study by Hallett et al. (2004). Our preliminary study also indicated that the spotty population seem to be more active in feeding and mating compared to stripe population. We would like
Serangga

to target the salivary gland of RPW in order to inactive its feeding system as a way of helping integrated pest management (IPM) in future. For this study, two biogenic amines, dopamine and serotonin were used as a marker. Both amines have been found to regulate the salivary gland of insects (Ali, 1997b). Our focus is to determine the presence and distribution of serotonin and dopamine in the salivary gland of RPW. The presence of these amines would lead to explain the roles of biogenic amines (dopamine and serotonin) in salivary glands of the RPW. The presence of these amines in the nerves fibers within the salivary gland suggests that these amines control the salivary glands as neurotransmitters.

Samples were collected from 7 districts in Terengganu namely Besut, Setiu Kuala Terengganu, Marang, Hulu Terengganu, Dungun and Kemaman. The study area was mainly located at coastal areas and in villages where coconut trees are easily found and also in few FELDA and FELCRA oil palm estates in Terengganu. Samples of RWP were also obtained from Crop Protection and Plant Quarantine Davison, Department of Agriculture, Kuala Terengganu. RPW were dissected to collect the salivary glands for ex-situ analysis by immunohistochemistry (IHC) techniques.

In this study, IHC analysis revealed the distribution of serotonin and dopamine within the salivary gland of two morphologically distinctive RPW. Serotonin immune reactivity were detected on both of spotty (Fig. 1B) and stripe (Fig. 1C) RPW. This was shown from the intense staining of the fluorescence compared to the control image (Fig. 1A). For dopamine distribution, only spotty RPW showed the much intensity in FITC staining (Fig. 1D), while there is no obvious staining appeared in stripe RPW (Fig. 1E). The accumulation of serotonin-like immune reactivity on certain area of the salivary gland can be assumed that it may play role as a neurohormone, like in *Rhodnius*’s salivary gland (Orchard, 2005) *Locusta*’s alimentary tract (Molaie and Lange, 2003) where it will be release into the hemolymph. The salivary gland of both spotty and stripe RPW were probably innervated by neurohormonal activities of serotonin and dopamine as there were no evidence of staining on the nerves fibers as such they are not act as neurotransmitter.
Fig. 1. (A) Microphotographs of negative control of whole mount tissue of RPW salivary gland. (B) and (C) Bright staining of serotonin-like immunoreactivity in the salivary gland of spotty and stripe RPW (respectively). (D) Dopamine fluorescence staining on the salivary gland of spotty RPW. (E) No dopamine-like immunoreactivity on the salivary gland of stripe RPW.
In general, the minor differences of biogenic amine expression in both spotted and striped RPW had supported the study by Hallet et al (2004) when both RPW variation had been synonymized as *Rhynchophorus ferrugineus*. This study shows that 5HT & DA are involved in the regulations of the SG that the understanding of salivary gland control and saliva formation in the RPW and in other insects need further investigation.

Internal morphology and physiology on salivary glands and digestive systems RPW that invaded Terengganu have not been documented so far. Understanding on how the salivary gland regulates during feeding will lead to a better control on the mechanism pathway of salivation by targeting specific organ/system. More importantly is that this study could be a starting point for future development of new specific pesticides targeting on the dopamine and serotonin involvement in feeding and digestion process in RPW. This valuable finding would be a great help in integrated pest management (IPM) program of RPW and that ensure the sustainability of oil palm production in Malaysia.

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