Serangga 18(1): 77-87
ISSN 1394-5130 © 2013, Centre for Insect Systematics,
Universiti Kebangsaan Malaysia

SHORT COMMUNICATIONS

A BIOGENIC AMINE EXPRESSION IN THE SALIVARY GLAND OF TWO MORPHOLOGICALLY DISTINCTIVE RED PALM WEEVIL, *RHYNCHOPHORRUS FERRUGINEUS* (OLIVIER, 1790) (COLEOPTERA: CURCULIONIDAE)

[1]Nurul Hidayah A. S., Nurul Wahida O.*,
[2]Norefrina Shafinaz M. D. &[1]Idris. A. B.
[1]School of Environmental and Natural Resource Sciences,
Faculty Science and Technology
Universiti Kebangsaan Malaysia, Bangi, 43600, Selangor
[2]School of Bioscience and Biotechnology,
Faculty Science and Technology
Universiti Kebangsaan Malaysia, Bangi, 43600, Selangor
Corresponding author: nwahida.othman@gmail.com

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 weevilis a very destructive insect pest to plants under the family of Palmaceae 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 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 glandof 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.

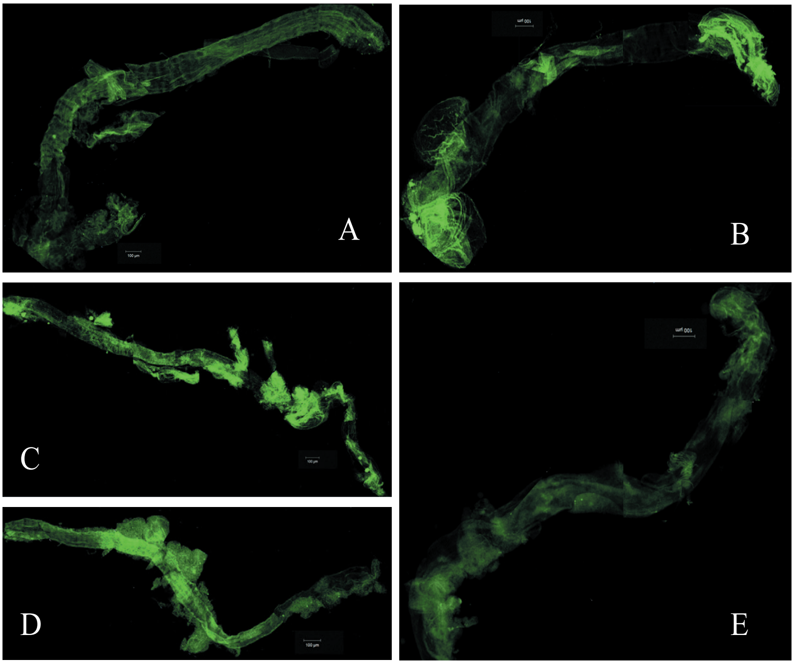

Fig. 1. (A) Microphotographs of negative control of whole mount tissue of RPW salivary gland. (B) and (C) Bright staining of serotonin-like immunoreactivity in the salivary gland of spotty and stripe RPW (respectively). (D) Dopamine fluorescence staining on the salivary gland of spotty RPW. (E) No dopamine-like 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 ferrugeniues*. 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.

ACKNOWLEDGEMENT

This work is financially supported by GUP grant (GUP-2012-086) from Universiti Kebangsaan Malaysia (UKM) and FRGS grant (FRGS/1/2012/ST03/UKM/03/1/STWN) from Minister of Higher Education. Special thanks goes to Dr. Wahizatul from Universiti Malaysia Terengganu, Mr. Zazali from Department of Agriculture, Terengganu and Mr. Ruslan Md. Yusop (UKM) for helping us with the RPW samples.

REFERENCES

Abe F., Hata K., and Sone K., (2009). Life history of the red palm weevil, *Rhynchophorus ferrugineus* (Coleoptera: Dryophtoridae), in Southern Japan. *Florida Entomologist* 92 (3), 421-425.

Abozuhairah, R.A., Vidyasagar, P.S.P.V., Abraham, V.A., 1996. Proceedings of the XX International Congress of Entomology, Firenze, Italy, August 25-31, pp. 541.

Ali, D. W. 1997a. On the aminergenic innervations of locust (*Locusta migrutoria*) salivary glands. *National Library of Canada.* 240

Ali, D. W. 1997b. The aminergic and peptidergic innervations of insect salivary glands. *The Journal of Experimental Biology.* 200: 1941-1949

Ali, D., W., and Orchard, I. 1996. The uptake and release of serotonin and dopamine associated with locust (*Locusta migratoria)* salivary glands. *The Journal of Experimental Biology.* 199: 699-709

Basari N., Azmi W.A., Chik Z., and Abdul Razak A.R. (2011). Coconut Pest Alert! Onslought of Coconut Palms by the lethal Pest Red Palm Weevil, *Rhynchophorus ferrugineus*, in Terengganu. *INFOKUS,* Universiti Malaysia Terengganu, Bil. 35. June 2011.

Baumann A, Blenau W, Erber J. 2003. Biogenic amines. In: Resh VH, Cardé RT, editors. *Encyclopedia of Insects*. San Diego: Academic Press. p 91-94.

Baumann, O., D. Kuhnel, et al. (2004). Dopaminergic and serotonergic innervation of cockroach salivary glands: distribution and morphology of synapses and release sites. *Journal of Experimental Biology* 207(15): 2565-2575.

Bernama. 2010. Minyak Sawit menyumbang RM 178b kepada ekonomi Malaysia pada tahun 2020. Bernama. Retrieved 29 September 2012, from http: //web6.bernama.com/bernama/v3/newslite.php?.id=549603.

Cook, H. and I. Orchard. 1990. Effects of 5,7-DHT upon feeding and serotonin content of various tissues in *Rhodnius prolixus*. *Journal of Insect Physiology*. 36(5): 361-363, 365-367.

Dutta R., Thakur N., S., A., Bag T.S., Anita N., Chandra S., and Ngachan. 2010. New record of red palm weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) on Arecanut (*Areca catechu*) from Meghalaya, India. *Florida Entomologist* 93 (3), 446-448

Elia, A. J., D. W. Ali and I. Orchard. 1994."Immunochemical Staining of Tyrosine-Hydroxylase (Th)-Like Material in the Salivary-Glands and Ventral Nerve Cord of the Cockroach, *Periplaneta americana* (L). *Journal of Insect Physiology*. 40(8): 671-683.

Faleiro, J. R. 2006. A review of the issues and management of the red palm weevil *Rhynchophorus ferrugineus* (Coleoptera: Rhynchophoridae) in coconut and date palm during the last one hundred years. *International Journal of Tropical Insect Science*, 26(03), 135-154.

Hallett, R. H., Crespi, B. J., & Borden, J. H. 2004. Synonymy of *Rhynchophorus ferrugineus* (Olivier), 1790 and *R. vulneratus* (Panzer), 1798 (Coleoptera, Curculionidae, Rhynchophorinae). *Journal of Natural History*. 38(22): 2863-2882

House, C. R. 1980. Physiology of Invertebrate Salivary-Glands. *Biological Reviews of the Cambridge Philosophical Society*55 (NOV): 417-473.

House CR, Ginsborg BL. 1985. Salivary gland. In: Kerkut GA, Gilbert LI, editors. Comprehensive insect physiology, biochemistry and pharmacology, Vol. 11. Oxford: Pergamon Press. p 195-224.

Ju, R. T., Li, Y. Z., Du, Y. Z., Chi, X. Z., Yan, W., and Xu, Y. 2006. Alert to spread of an invasive alien species, red palm weevil, *Rhynchophorus ferrugineus*. *Chinese Bull. Entomol*, 43(2), 159-163.

Just, F. and B. Walz. 1994. Salivary glands of the cockroach, *Periplaneta americana*: New data from light and electron microscopy. *Journal of Morphology*. 220(1): 35-46.

Just, F. and B. Walz. 1996. The effects of serotonin and dopamine on salivary secretion by isolated cockroach salivary glands. *Journal of Experimental Biology* 199(2): 407-413.

Kendall, M. D. 1969. Fine Structure of Salivary Glands of Desert Locust Schistocerca Gregaria Forskal. *Zeitschrift Fur Zellforschung Und Mikroskopische Anatomie* 98(3): 399

Lang, I. and B. Walz. 1999. Dopamine stimulates salivary duct cells in the cockroach *Periplaneta americana*. *Journal of Experimental Biology*. 202(6): 729-738.

Li Y., Zhu Z.R., Ju R., and Wang L.S., 2009. The red palm weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae), Newly reported from Zhejiang, China and update of geographical distribution. *Florida Entomologist*, 92(2), 386-387 *Molecular & Integrative Physiology* 145(1): 114-122.

Marg, S., B. Walz and W. Blenau. 2004. The effects of dopamine receptor agonists and antagonists on the secretory rate of cockroach (*Periplaneta americana*) salivary glands. *Journal of Insect Physiology* 50(9): 821-830.

Miggiani, L., I. Orchard and V. TeBrugge 1999. "The distribution and function of serotonin in the large milkweed bug, *Oncopeltus fasciatus*: a comparative study with the blood-feeding bug, *Rhodnius prolixus*. *Journal of Insect Physiology* 45(11): 1029-1036.

Molaei, G. & Lange, A. B. 2003. The association of serotonin with the alimentary canal of the African migratory locust, *Locusta migratoria*: distribution, physiology, and pharmacological profile. *Journal of Insect Physiology* 49: 1073-1082.

Murphy S. T., and Briscoe B.R., 1999. The red palm weevil as an alien invasive: biology and the prospects for biological control as a component of IPM. *Biocontrol News and Information,* 20.1., 35-46

Orchard, I. and V. T. Brugge. 2002. Contractions associated with the salivary glands of the blood-feeding bug, *Rhodnius prolixus*: evidence for both a neural and neurohormonal coordination. *Peptides* 23(4): 693-700.

Orchard, I. 2006. Serotonin: A coordinator of feeding-related physiological events in the blood-gorging bug, Rhodnius prolixus. *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology* 144(3): 316-324.

Roda A., Kairo M., Damian T., Franken F., Heidweiller K., Johanns C., and Mankin R.. 2011. Red palm weevil (*Rhynchophorus ferruginues*), an invasive pest recently found in the Caribean that threatens the region. *EPPO Bulletin*. 41, 116-121

Roeder, T. 1994. Biogenic amines and their receptor in insects. *Comparative Biochemistry and Physiology.* 107C:1-12

Sekhar, I. 2000. Titanic loss from a tiny weevil in coconut. *Indian Coconut J.*, 30 (9), pp. 8-10

Walz, B., O. Baumann, et al. 2006. The aminergic control of cockroach salivary glands. *Archives of Insect Biochemistry and Physiology* 62(3): 141-152. waterbugs (Heteroptera; Belostomatidae). *Comparative Biochemistry and Physiology - Part A:*

Watanabe, H. and M. Mizunami. 2006. Classical conditioning of activities of salivary neurones in the cockroach. *Journal of Experimental Biology.* 209(4): 766-779

Wattanapongsiri A., 1966. A revision of the genera *Rhynchophorus* and dynamis (Coleoptera:Curculionidae). *Department of Agriculture Science Bulletin 1* (Bangkok: (Bangkok: Department of Agriculture) 1-418

Zimmermann B, Walz B. 2003. Hormone mediated intercel- lular calcium signalling in an insect salivary gland: path- ways and mechanisms. In: Falcke M, editor. Understanding calcium dynamics. Berlin: Springer-Verlag. p 119-129.