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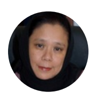

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Foes can be Friends: Laboratory Trials on Invasive Apple Snails, *Pomacea* spp. Preference to Invasive Weed, *Limnocharis flava* (L.) Buchenau Compared to Rice, *Oryza sativa* L.

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A B S T R A C T

Rice ecosystem often involves a complex interaction of various pest species with rice. An example of such interaction is that between the invasive *Pomacea* spp. and *Limnocharis flava*. Both invasive pests have caused heavy loss to rice in Southeast Asia including Malaysia. However, information on the interaction of both invasive pests in the rice field is still lacking. Therefore, a laboratory study was conducted to determine the feeding preference and growth pattern of *P. canaliculata* and *P. maculata* for rice and *L. flava* based on choice and no-choice experiments. It was found that the three different sizes (1, 2 and 3 cm) of both *Pomacea* spp. used in this study showed significantly less preference for rice stems and leaves as compared to *L. flava* leaves and stems. Among all the studied plant materials, rice stems were the least consumed. Moreover, higher shell length growth and weight gain was also recorded in the two species when fed with *L. flava*. This further suggests that the two *Pomacea* spp. have the potential to be used as an effective bio-control agent against the invasive weed in the rice ecosystem during the early stages of rice growth, in combination with a proper management of irrigation water. In addition, *L. flava* can also be used as a trap crop in rice fields to ease the collection and destruction of apple snails.

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Authors' Contributions
AAG, RM, DO and NAAZ conceived and designed the study. AAG performed the experiment work. AAG and MG analyzed the data and wrote the article.

Key words
Invasive, apple snails, *Pomacea*, *Oryza sativa*, feeding preference, *Limnocharis flava*.

INTRODUCTION

Many species of apple snails (*Pomacea* spp.) were introduced to Southeast Asian countries from their native habitats of South America for aquarium and food trade (Hayes *et al*., 2008; Nghiem *et al*., 2013). Among these species, *Pomacea canaliculata* and *Pomacea maculata* are dominant and widely distributed (Hayes *et al.*, 2008; Arfan *et al.*, 2014). Both species have caused significant losses to many macrophytes and agricultural crops, especially rice in Southeast Asia and taro in North America (Horgan *et al.*, 2014). Although, *Pomacea* spp. were known to be non-damaging at their native habitat, the estimated losses incurred to rice in invaded countries of Southeast Asia were billions of dollars (Joshi, 2007; Hayes *et al*., 2008; Nghiem *et al*., 2013).

Pomacea spp., although omnivorous in feeding habits are predominantly phytophagous; often causing heavy losses to cultivated crops including rice (Burlakowa *et al.*, 2009). *Pomacea* spp. are extremely damaging to young rice seedlings due to their soft and succulent parts (Sanico *et al.*, 2002; Horgan *et al.*, 2014). Substantial damage by snails could cause the complete loss of rice crop; however this further depends upon the size and density of snails, stage of rice crop and availability of water (Teo, 2003). The availability of many macrophytes that includes weeds and other autotrophs along with continuous presence of water makes the rice ecosystem suitable for the growth and development of apple snails (Sanico *et al*., 2002).

Other major constraints to rice yield are rice weeds. Composed of around 140 species; weeds often causes yield losses up to 50% (Rao *et al.*, 2007; Anwar *et al.*, 2011). Apart from the important *Oryza sativa* L. (weedy rice), *Echinochloa* spp., *Leptochloa chinensis* (L.) Nees, *Fimbristylis miliacea* (L.) Vahl. and *Limnocharis flava* (L.) Buch. are among the major weeds reported in Malaysia (Karim *et al.*, 2004). Yellow bur-head, *Limnocharis flava* L. is a perennial broad leaf weed often found in rice fields. It originates from South and Central American countries, the region from where *Pomacea* spp. also originated. Currently, it is widely distributed in Southeast Asia including Malaysia and Australia, and it is regarded as a damaging weed in rice fields and other aquatic habitats (Juraimi *et al.*, 2012). Apart from being a damaging weed, it has been incorporated for vegetable and ornamental purposes, thus further supporting its

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establishment in the local ecosystem (Waterhouse, 2003).

To date various aspects of *Pomacea* spp. have been studied, for example population distribution (Arfan *et al.*, 2014), density dependence (Yoshida *et al*., 2013) and biological aspects of mating and reproduction (Tamburi and Martin, 2009; Arfan *et al.,* 2015). However, only a few studies have highlighted the possible role of apple snails as weed control agents in rice (Yusa *et al*., 2003; Joshi *et al*., 2005). Supporting studies also lack on the effect of various rice weeds on the feeding, growth and other biological aspects of *P. canaliculata* and *P. maculata*. Recent studies by Juraimi *et al. (*2012) has also highlighted that *L. flava* has developed resistance against many available herbicides, and further suggested for alternate control measures. Considering the above knowledge gaps and the necessity to identify optional control procedures for apple snails and weed, this study was undertaken to understand the feeding preference of different sizes of *P. maculata* and *P. canaliculata* on rice and *L. flava* and their effects on the growth and weight gain of the *Pomacea* spp. The results obtained will be helpful in the management of the invasive *Pomacea* spp. by cultivating *L. flava* as trap crop in rice fields or management of *L. flava* and other likewise broad leaf weeds in rice and other aquatic habitats through proper utilization of *Pomacea* spp.

MATERIALS AND METHODS

Pomacea *snails*

Three different sizes of *P. maculata* and *P. canaliculata* (1, 2 and 3 cm) were selected for the study considering their variable damage potential to rice. Egg masses from the two *Pomacea* spp. cultures maintained at laboratory were taken and placed in separate Petri dishes. Algae, *Scenedesmus* sp., were provided as food for the freshly hatched hatchlings, whereas rice and *L. flava* were provided to them during fourth week of their development. To avoid any bias, snails of same age and same egg masses were used in the study. The age of 1, 2 and 3 cm snails used in the study was 4, 7 and 11 months, respectively. All snails were kept on starvation for 24 hours before the feeding trials.

Plant materials

Rice, *Oryza sativa* and *L. flava* were cultivated at Glass House, Faculty of Agriculture, Universiti Putra Malaysia, Malaysia. Three-week old plants were used in the experiments as snails are more damaging to young rice seedlings in comparison to older rice plants. Leaves and stems of the two plants were used in the study as snails feed only on the above ground plant parts (Arfan, 2015). Three grams of each plant material were provided as fresh food in individual replicate on a daily basis.

No-choice experiment

In a no-choice experiment, leaves and stems of rice and *L. flava* were provided to different sizes of apple snails separately in the plastic containers. Treatments for the experiment were: T_1= rice leaves (RL), T_2 = rice stems (RS), T_3 = *L. flava* leaves (YL) and T_4 = *L. flava* stems (YS). Five replications of each treatment were maintained.

Choice experiment

Different combinations of rice and *L. flava* were provided to various sizes of apple snails to assess their preferences and effects on their growth and weight gain. Treatments for choice experiments were T_1 = Rice leaves + Rice stems (RLS), T_2 = Rice leaves + *L. flava* stems (RLYS), T_3 = Rice leaves + *L. flava* leaves (RLYL), T_4 = Rice stems + *L. flava* leaves (RSYL), T_5 = Rice stems + *L. flava* stems (RSYS) and T_6 = *L. flava* leaves + *L. flava* stems (YBH). Five replications of each plant material were maintained.

Experimental setup, data collection and statistical analysis

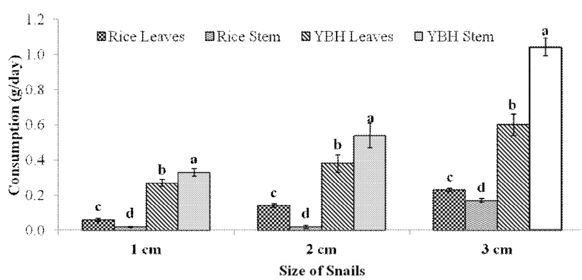
Five replications per treatment were maintained in choice and no-choice experiments. In each replicate, two snails of the same size and same species were introduced. Tap water at a level of 5 cm was maintained in the containers (20x10x10 cm) and changed on a daily basis. Observations were taken daily for seven days for all the treatments simultaneously. Blotting papers were used for removing the water from plants and snails before and after feeding trials. Digital balance (AE 240, Mettler Toledo, USA) was used for taking the weight of snails and plant materials, whereas digital Calipers (Mitutoyo, USA) were used for the measurement of shell length of snails. The same quantity of all the plant materials was kept separately in the plastic containers to assess water absorption and the same was adjusted accordingly to calculate the net feeding by the snails. The experiment was conducted in a Completely Randomized Design. One-way Analysis of Variance was used to analyze all the collected data, except for mean daily consumption in a choice experiment. The significant mean differences were compared using Least Square Difference at 0.05 probability level. In the choice experiment, mean daily consumption of plant materials by *P. maculata* and *P. canaliculata* was analyzed using Student t-test at 0.05 probability level. All the analyses of the obtained data were performed using SAS version 9.3 (SAS Institute Inc. 2009).

RESULTS

Results of the choice and no-choice experiments confirmed higher preference of *P. canaliculata* and *P. maculata* for *L. flava* in comparison to rice along with weight gain and shell length growth.

No-choice experiment

The results regarding the consumption of leaves and stems of rice and *L. flava* by different sizes of *P. canaliculata* and *P. maculata* are shown in Figure 1. Significantly higher consumption by 1 cm *P. canaliculata* and *P. maculata* were observed on *L. flava* stems (F=71.7; DF=3; P<0.05 and F=115.3; DF=3; P<0.05, respectively). Similarly, 2 cm and 3 cm *P. canaliculata* and *P. maculata* also showed significantly higher preference for *L. flava* stems followed by *L. flava* leaves (F=52.1; DF=3; P<0.05 and F=31.5; DF=3; P<0.05, and F=91.9; DF=3; P<0.05 and F=109.0; DF=3; P<0.05, respectively).



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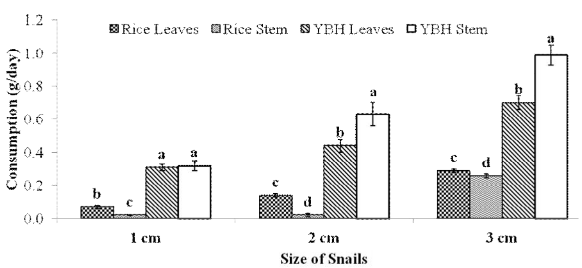

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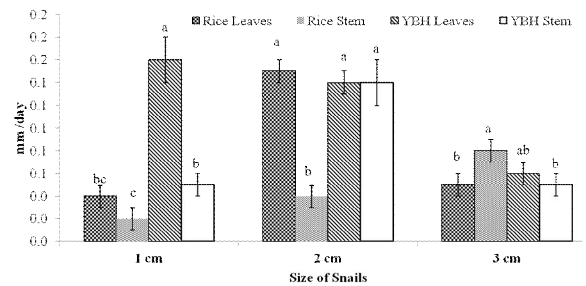
Fig. 1. Consumption of rice and yellow bur-head (g/day) by *P. maculata* (A) *P. canaliculata* (B) in no-choice experiment.
Means ± SE followed by the same letters against each snail size are not significantly different (LSD, P < 0.05).

The significantly higher shell length growth in 1 cm *P. canaliculata* and *P. maculata* were recorded on *L. flava* leaves (F=53.2; DF=3; P<0.05 and F=39.1; DF=3; P<0.05, respectively). Significantly higher growth in 2 cm *P. canaliculata* was also recorded on *L. flava* leaves (F=40.8; DF=3; P<0.05), whereas 2 cm *P. maculata* showed higher growth on rice leaves but was not significantly different from *L. flava* leaves and *L. flava* stems (F=0.09; DF=3; P>0.05). Significantly the least growth in 3 cm *P. canaliculata* was recorded on rice stems (F=5.5; DF=3; P<0.05), whereas no significant difference was observed in other treatments. However, 3 cm *P. maculata* showed the highest growth on rice stems but was not significantly different in *L. flava* leaves (Fig.2).



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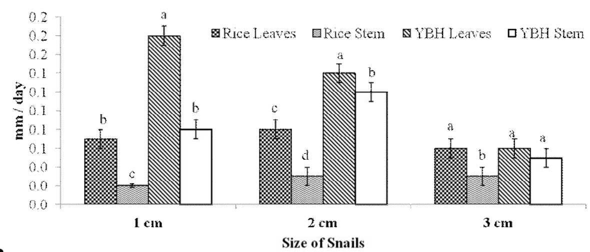

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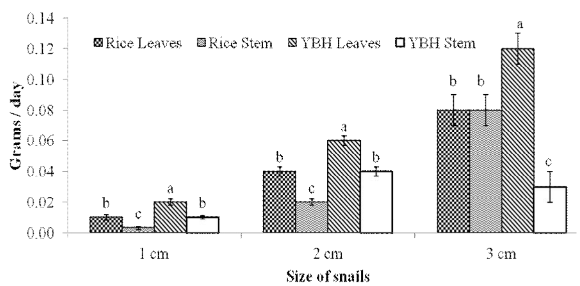
Fig. 2. Shell length growth (mm/day) of *P. maculata* (A) and *P. canaliculata* (B) feeding on yellow bur-head and rice in no-choice experiment.
Means ± SE followed by the same letters against each snail size are not significantly different (LSD, P < 0.05).

Results showing the weight gain by the two species confirmed that significantly higher weight gain by all the three sizes of *P. canaliculata* and *P. maculata* was recorded on *L. flava* leaves (P<0.05), whereas the least weight gain was either recorded on rice stems or leaves (Fig. 3).

Choice experiment

Table I highlights the daily consumption by different sizes of *P. canaliculata* and *P. maculata* on rice and *L. flava* leaves and stems in choice experiments. In choice experiment of rice leaves and stems, all sizes of the two snail species showed significantly more preference for rice leaves compared with rice stems (P<0.05). Meanwhile, *L. flava* stems or leaves were significantly more preferred by all three sizes of *P. canaliculata* and *P. maculata* when provided with either rice leaves or stems in choice experiments, except for 3 cm *P. canaliculata* who showed similar preference for rice leaves and *L. flava* stems. Results also confirmed that 1 cm snails of *P. canaliculata* and *P. maculata* showed significantly more preference for *L. flava* leaves in comparison to *L. flava* stems (t=5.3; DF=12; P<0.05 and t=2.7; DF=12; P<0.05, respectively). No significant difference between *L. flava* leaves and stems was observed for 2 cm snails of the two species (t=1.7; DF=12; P>0.05 and t=0.3; DF=12; P>0.05), whereas 3 cm snails of *P. canaliculata* and *P. maculata* showed significantly more preference for *L. flava* stems as compared to leaves (t=2.4; DF=12; P<0.05 and t=3.5; DF=12; P<0.05, respectively).



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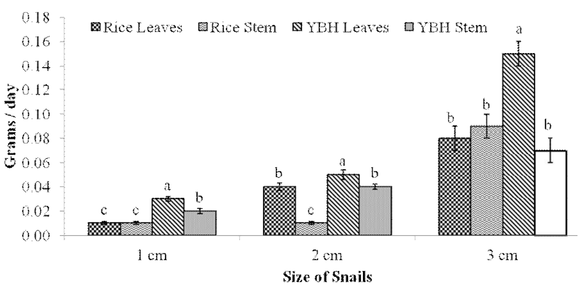

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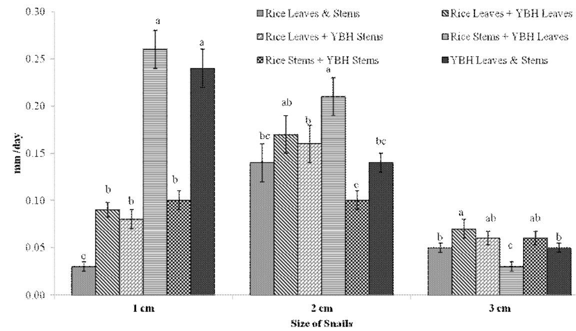
Fig. 3. Weight gain (g/day) of *P. maculata* (A) and *P. canaliculata* (B) feeding on yellow bur-head and rice in no-choice experiment.
Means ± SE followed by the same letters against each snail size are not significantly different (LSD, P < 0.05).

The results for the shell length growth in 1, 2 and 3 cm snails *P. canaliculata* and *P. maculata* as given in Figure 4 and showed that 1 cm *P. canaliculata* grew significantly more when provided with a combination of *L. flava* leaves and stems (F=46.2; DF=5; P<0.05). However, 1 cm *P. maculata* grew significantly more in combination of *L. flava* leaves with rice stems (F=44.5; DF=5; P>0.05) but was not significantly different when feeding with a combination of *L. flava* leaves and stems. Similar results were obtained for 2 cm *P. canaliculata* and *P. maculata* that showed significantly more growth



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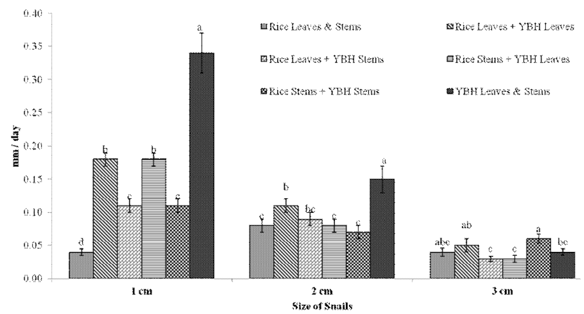

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Fig. 4. Shell length growth (mm/day) of *P. maculata* (A) and *P. canaliculata* (B) feeding on yellow bur-head and rice (mm / day) in choice experiment. Means ± SE followed by the same letters against each snail size are not significantly different (LSD, P < 0.05).

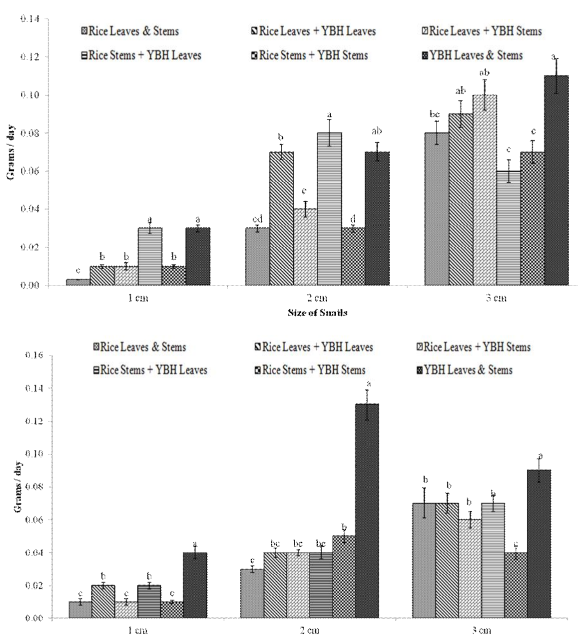
in combination of *L. flava* leaves with stems and rice stems with *L. flava* leaves (F=7.5; DF=5; P<0.05 and (F=4.6; DF=5; P>0.05, respectively). Although, 3 cm *P. canaliculata* showed more growth in combination of rice stems with *L. flava* stems but it was not significantly different when feeding on combination of rice leaves with *L. flava* leaves or *L. flava* leaves with stems (F=0.001; DF=5; P>0.05). However, 3 cm *P. maculata* showed more growth when fed with a combination of rice leaves and *L. flava* leaves but it was not significantly different from the growth recorded in the combination of either rice leaves with *L. flava* stems and rice stems with *L. flava* stems (F=0.004; DF=5; P>0.05).

The results regarding the weight gained by 1, 2 and 3 cm size snails *P. canaliculata* and *P. maculata* in choice experiments showed that except 2 cm *P. maculata,* all the other sizes of *P. maculata* and *P. canaliculata* gained significantly more weight when fed with a combination of *L. flava* leaves and stems (P<0.05; Fig. 5). The 2 cm *P. maculata* showed more weight gain when fed with a combination of rice stems and *L. flava* leaves but was not significantly different, when fed with a combination of *L. flava* leaves and stems (F=0.01; DF=5; P>0.05).

Table I.- Consumption of rice and *L. flava* by *P. canaliculata* and *P. maculata* (grams / day) in choice experiment.

Treatment	1 cm		2 cm		3 cm	
	P. canaliculata	***P. maculata***	***P. canaliculata***	***P. maculata***	***P. canaliculata***	***P. maculata***
T_1	0.08 ± 0.01^{a}	0.06 ± 0.01^{a}	0.12 ± 0.01^{a}	0.13 ± 0.01^{a}	0.21 ± 0.01^{a}	0.23 ± 0.01^{a}
	0.02 ± 0.01^{b}	0.01 ± 0.004^{b}	0.01 ± 0.003^{b}	0.00^{b}	0.12 ± 0.01^{b}	0.05 ± 0.01^{b}
T_2	0.04 ± 0.01^{b}	0.04 ± 0.01^{b}	0.13 ± 0.01^{b}	0.14 ± 0.01^{b}	0.24 ± 0.01^{a}	0.27 ± 0.01^{b}
	0.22 ± 0.02^{a}	0.21 ± 0.01^{a}	0.29 ± 0.03^{a}	0.36 ± 0.04^{a}	0.28 ± 0.03^{a}	0.34 ± 0.03^{a}
T_3	0.06 ± 0.01^{b}	0.05 ± 0.01^{b}	0.16 ± 0.04^{b}	0.12 ± 0.01^{b}	0.24 ± 0.01^{b}	0.21 ± 0.01^{b}
	0.29 ± 0.02^{a}	0.23 ± 0.01^{a}	0.32 ± 0.04^{a}	0.30 ± 0.04^{a}	0.40 ± 0.04^{a}	0.46 ± 0.04^{a}
T_4	0.01 ± 0.004^{b}	0.02 ± 0.01^{b}	0.02 ± 0.01^{b}	0.02 ± 0.01^{b}	0.17 ± 0.02^{b}	0.16 ± 0.02^{b}
	0.20 ± 0.03^{a}	0.28 ± 0.02^{a}	0.30 ± 0.03^{a}	0.39 ± 0.05^{a}	0.51 ± 0.05^{a}	0.61 ± 0.06^{a}
T_5	0.01 ± 0.004^{b}	0.01 ± 0.003^{b}	0.01 ± 0.01^{b}	0.02 ± 0.01^{b}	0.16 ± 0.01^{b}	0.13 ± 0.02^{b}
	0.16 ± 0.02^{a}	0.25 ± 0.02^{a}	0.37 ± 0.04^{a}	0.51 ± 0.05^{a}	0.53 ± 0.06^{a}	0.62 ± 0.07^{a}
T_6	0.29 ± 0.02^{a}	0.21 ± 0.02^{a}	0.39 ± 0.05^{a}	0.31 ± 0.04^{a}	0.47 ± 0.04^{b}	0.43 ± 0.04^{b}
	0.05 ± 0.01^{b}	0.10 ± 0.02^{b}	0.30 ± 0.04^{a}	0.28 ± 0.05^{a}	0.70 ± 0.07^{a}	0.57 ± 0.04^{a}

Means ± SE in treatment for same size of individual species with different letters differ significantly ($P < 0.05$)
T_1, Rice leaves + Rice stems (RLS); T_2, Rice leaves + *L. flava* stems (RLYS); T_3, Rice leaves + *L. flava* leaves (RLYL); T_4, Rice stems + *L. flava* leaves (RSYL); T_5, Rice stems + *L. flava* stems (RSYS) and T_6, *L. flava* leaves + *L. flava* stems (YBH).



A

B

Fig. 5. Weight gain (g/day) *P. maculata* (A) and of *P. canaliculata* (B) feeding on yellow bur-head and rice in choice experiment.
Means ± SE followed by the same letters against each snail size are not significantly different (LSD, $P < 0.05$).

DISCUSSION

The results of choice and no-choice experiments performed under laboratory conditions revealed that all sizes of snail species *i.e.*, *P. maculata* and *P. canaliculata* showed significantly higher preferences for leaves and stems of *L. flava* in comparison to *O. sativa*. Both the snail species also showed higher growth in shell length and weight gain when they were fed with leaves and stems of *L. flava*. The lower preference and feeding on rice compared to weeds, *Alternanthera philoxenoides* and *Monochoria vaginalis* by *P. canaliculata* were also reported by Zhao *et al.* (2012). Higher preference of both species towards *L. flava* may be due to the higher nutritional value in its soft and succulent leaves and stems along with less physical and chemical defenses in the plant. Studies confirmed that nutritional value and chemical composition of plants exhibited significant role in the feeding of *Pomacea* spp. as snails showed more invasiveness on plants with less chemical and physical contents but rich in nutrients (Burlakova *et al.*, 2009; Baker *et al.*, 2010; Wong *et al.*, 2010; Morrison and Hay, 2011; Qiu *et al.*, 2011). *Pomacea* spp. had previously showed less preference to plants that possessed higher quantities of phenol or other chemicals that deter their feeding, growth and survival (Wong *et al.*, 2010; Qiu *et al.*, 2011). Test has revealed that rice plants contain higher amount of insoluble phenols as compared to soluble phenols with ferulic acid and p-coumaric acid as major insoluble phenols and 6'-*O*-(*E*)-Feruloylsucrose and 6'-*O*-(*E*)-sinapoylsucrose as major soluble phenols, thus supporting the lack of preference towards rice in comparison to *L. flava*. (Tian *et al.*, 2004). Therefore, higher preference shown by *P. canaliculata* and *P. maculata* towards *L. flava* may be because of its tenderness and more nutritional value as compared to rice along with less phenolic compounds and chemical defenses.

Based on this laboratory assay, we have identified a higher preference of *P. maculata* and *P. canaliculata* towards *L. flava* in comparison to *O. sativa*. This finding further opens the path towards understanding the possibility of incorporating and manipulating both *Pomacea* spp. and *L. flava* as bio-control agents that complement each other, thus leading to a well managed rice field. However, under field conditions, feeding preference of *Pomacea* spp. may be influenced by the stage of rice crop and diversified weeds. Generally the snails are most destructive to direct seeded and transplanted rice up to the fourth and third week respectively (Sanico *et al*., 2002; Teo, 2003; Wada, 2004). Therefore, in this study using the three week old plants which are often the target of *Pomacea* spp.; we managed to highlight that the snails preferred *L. flava* when presented with and option of both plant. Moreover, *L. flava* has also been reported as one of the five major weeds recorded in Malaysia (Karim *et al.,* 2004). As this weed is often omnipresent in the rice fields, the experiments that have been conducted in this study are crucial in understanding its interaction with *Pomacea* spp.

To date, farmers often resort to chemicals for the control of invasive *Pomacea* spp. and *L. flava*, however, the available chemicals often do not provide effective control of both snails and *L. flava* either due to inefficiency of chemicals or the development of resistance against them (Schnorbach *et al*., 2006; Juraimi *et al.,* 2012). Cultural, mechanical and biological control measures have been suggested as preferable options to control these two invasive pests of rice (Juraimi *et al.,* 2012; Liang *et al.,* 2013). Accordingly, the use of both *Pomacea* spp. as potential bio-control agents in rice fields not only against *L. flava* but also against other major weeds could be evaluated as snails showed more preference to *L. flava* than rice in this study. Moreover, *L. flava* could also be exploited as a trap crop to facilitate easy picking and destruction of apple snails in rice fields. However, further quantitative studies should be conducted under field conditions to evaluate the role of *Pomacea* spp. in regulating the population of rice weeds with ultimate influence on the rice yield.

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Statement of conflict of interest

There was no conflict of interest from the authors.

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