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SOWING METHODS AND WATER LEVELS INFLUENCE APPLE SNAIL DAMAGE TO RICE AND ITS YIELD IN PENINSULAR MALAYSIA

By A. G. ARFAN^{†‡}, R. MUHAMAD^{†§}, D. OMAR[†], A. A. NOR AZWADY[¶]
and G. MANJERI[†]

[†]*Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia,* [‡]*Department of Entomology, Faculty of Crop Protection, Sindh Agriculture University, Tando Jam, Sindh, Pakistan and* [¶]*Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*

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SUMMARY

Rice productivity is limited by many pests, especially *Pomacea* spp. in Southeast Asia. *Pomacea* spp. damage to rice depends on sowing methods, flooded conditions, and snail densities in the field. Therefore, this study aims to evaluate the effect of different sowing methods, water levels, and snail density (1, 2, and 3 snails per plot) on the damage potential of *Pomacea maculata* and *Pomacea canaliculata* to rice and its yield. Both species caused complete loss of crop in direct seeding and 14 days old transplanted rice. The least damage by both species was recorded in 21 and 28 days old transplanted rice with no further damage after week five. Irrigation and snail density also influenced damage whereby highest damage was recorded in rice grown with 5 cm water level in comparison to 2 cm. At 2 cm water level, damage by various snail densities was trivial. However, in 5 cm water level, damage increased with the increasing snail density and the highest damage was observed at three snails per plot of either species. No difference in inflicted damage to various treatments was observed between two species, suggesting their equal damage potential on rice. Meanwhile, rice yields in 2 cm water level treatments were compatible with 5 cm control treatment. The least yield was recorded in treatments with three snails per plot of either species at 5 cm water level. Understanding the effect of sowing method and suitable water level is important as it can be further incorporated into rice cultivation practices to reduce damage of apple snails and ensure a high yield during harvest.

INTRODUCTION

Rice, *Oryza sativa* L., is traditionally considered a submerged crop that requires plenty of water during most of its growth period (FAO, 2004). Due to the flooded conditions, transplanting is largely practiced in many areas of the world for rice cultivation. However, with the recent reduction in freshwater resources along with the increasing cost of labour, growers have shifted towards more water conserving rice cultivation techniques (Anwar *et al.*, 2010). This shift in techniques is based on the understanding that rice cultivated through these techniques can survive in water scarce environments with potential to produce yields comparable with transplanted rice (Ismail *et al.*, 2013). Another major issue in flooded rice cultivation is the presence of invasive apple snails,

[§]Corresponding author. Email: rita@upm.edu.my

Pomacea spp., that often cause complete loss of crop during its early development (Cowie, 2002; Teo, 2003). *Pomacea* spp., natives of South America, were introduced to many countries of the world, mainly for the aquarium and food businesses. But having failed to gain success, they were discarded in the wild and have since then become a serious threat to natural wetlands and cultivated crops (Arfan *et al.*, 2014; Horgan *et al.*, 2014; Rawlings *et al.*, 2007). Four apple snail species, i.e., *Pomacea canaliculata*, *Pomacea maculata*, *Pomacea diffusa* and *Pomacea scalaris* have been introduced into Southeast Asia with the former two being more abundant and widely distributed (Hayes *et al.*, 2008; Rawlings *et al.*, 2007). Higher abundance and wide distribution of *P. canaliculata* and *P. maculata* is attributed to their higher tolerance to environmental stresses and reproductive potential (Byers *et al.*, 2013; Kyle *et al.*, 2013; Hayes *et al.*, 2015). Rice being a flooded crop has been severely attacked by the both widely distributed and invasive *Pomacea* spp. in Southeast Asian countries where estimated yield losses can reach billions of dollars (Horgan *et al.*, 2014).

Considering the important role of water to rice cultivation and potential crop losses due to apple snail herbivory, this study was conducted to evaluate damage potential of *P. maculata* and *P. canaliculata* to rice based on two objectives. The first objective was to evaluate the damage potential of *Pomacea* spp. to rice grown by conventional methods and the second objective was to determine the effect of different water levels and densities of *Pomacea* spp. to damage on rice. Results obtained could be used to cultivate rice efficiently with an additional advantage of less damage by *Pomacea* spp.

MATERIAL AND METHODS

Damage potential of apple snails to rice grown by different methods

Study site. The study was conducted at the Serdang Selangor, Malaysia (2.9992°N, 101.7078°E) during February–May, 2014.

Snails. In this study, sexually adult snails of mixed sex (shell length = 3 cm) of *P. maculata* and *P. canaliculata* were used. All the snails were obtained from the laboratory reared culture of the two species obtained originally from field collections in Bukit Kechik, Kelantan, Malaysia (N05°50.942'E°102 29.353'). Identification of snails was done according to their shell morphology (Hayes *et al.*, 2012; Marwato and Nur, 2012), and final species confirmation was provided by Prof. Dr. R. H. Cowie.

Cultivation of rice. Rice variety MR219 was used in the experiment. Fourteen, 21 and 28 days old transplanted seedlings along with direct seeded rice were used in the experiment. Seedlings of different ages were established in plastic trays, so that they were ready to be transplanted on the same date for the experiment along with the direct seeded rice. For direct seeded rice, seed (5.16 g at 100 kg/ha) was soaked in water for 48 h and dried for 24 h before sowing for the experiment. Recommended NPK fertilizers were applied at the rate of 150:100:120 kg/ha at 15 days, 35 days, 55 days, and 80 days of the crop establishment in both transplanted and direct seeded rice.

Experimental setup. The rice cultivated by different methods was grown in individual plastic containers of $92 \times 61 \times 28$ cm³ size. Containers were filled with soil up to 15 cm to allow adequate root growth. Soaked seeds were directly sown in the containers for direct seeded rice, whereas seedlings of different ages were transplanted at two seedlings per hill (in rice hill means planting of two or more seedlings at one place) at spacing of 22×22 cm², resulting in 15 hills per container. Water in each container was maintained at 5 cm level. Two snails of each species were released separately in their respective treatment containers of rice cultivated by different methods. Control treatments of each sowing method without snails were also maintained to compare the effect of different mode of sowing on various agronomic components including yield with snail damaged treatments. Each treatment was replicated five times in a Completely Randomized Design. The study was conducted from sowing till harvesting of rice to compare the impact of damage of two *Pomacea* spp. on yield of rice cultivated via direct seeding and 14, 21, and 28 days transplanted seedlings.

Effect of various densities of apple snails to rice grown at two water levels

Study site. The study was conducted at Kampung Hutan Buloh (N 05°49.893'E102°43.4'), Melor, Kelantan, Malaysia during June–October, 2014.

Snails. Adult 3 cm sized snails of *P. maculata* and *P. canaliculata* were used in the study. Densities of snails used in the experiment were one, two, and three snails per plot.

Water levels. Two water levels were used in the experiment; flooded (5 cm) and saturated (2 cm). A water pump along with generator was used for maintaining the water levels in the individual treatments.

Land preparation and cultivation of rice. The field was divided into individual plots of 2×2 m². Each plot was separated by borders (15 cm high and 15 cm wide) along with fencing of 30 cm high wire mesh to restrict the entry of snails. Twenty eight days old transplanted rice seedlings of MR 219 were used in the experiment. Recommend NPK fertilizers at the rate of 150:100:120 kg was applied at 15 days, 35 days, 55 days, and 80 days. One day after transplanting, different densities of *P. maculata* and *P. canaliculata* (one, two, and three snails per plot) were released in their respective individual plots irrigated with water levels of 2 and 5 cm. Control plots of rice without snails at irrigation levels of 2 and 5 cm were also managed. Five replications of each treatment combination were maintained resulting in 70 rice plots in a Completely Randomized Design.

Data collection and analysis. Data collection begin one day after transplanting and continued on a weekly basis up to the maturity of crop. Data for percentage damage by *Pomacea* spp. in shape of missing hills was recorded. Information on number of tillers, number of panicles, and leaf area meter described as Leaf Area Index (LAI) was recorded at the time of flowering. Leaf area was measured using a Leaf Area

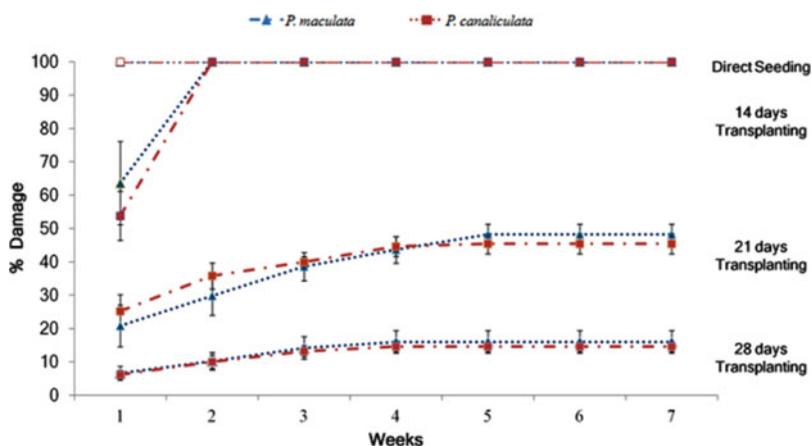


Figure 1. Weekly data on percentage missing hills of rice grown by different methods by *P. maculata* and *P. canaliculata*.

Meter (CI-203, CID Bio-Science, USA). The number of spikelets per panicle, grain filling percentage, 1000 grain weight, total dry weight, grain yield, and grain harvest index (GHI) were recorded at the time of harvesting. Entire rice plots were harvested for the yield data as missing hills were not uniformly distributed in the treatment plots and adjusted to 14% moisture. All the data obtained were in accordance with Sanico *et al.* (2002).

For the first experiment, Two-way Analysis of Variance (ANOVA) was used to analyse the collected data for different parameters as this experiment comprised of two factors, i.e., four sowing methods and two *Pomacea* spp. In the second experiment, Three-way Analysis of Variance (ANOVA) was used to analyse data as it was comprised of three factors, i.e., two *Pomacea* spp., two flood regimes, and three snail densities. In both experiments, Least Square Difference (LSD) at 0.05 probability level was used to compare means with significant differences. All the analyses were performed using Statistical Analysis System (SAS, version 9.3).

RESULTS AND DISCUSSION

Percentage damage

Results on percentage missing hills confirmed a significant difference among various conventional methods in respect to damage caused by two *Pomacea* spp. ($F = 954.20$, $n = 3$, $p < 0.001$; Figure 1). No difference in damage was observed between the two species individually ($F = 0.13$, $n = 1$, $p > 0.05$) or in combination within respective sowing methods ($F = 0.42$, $n = 3$, $p > 0.05$). Both species caused 100% damage to direct seeded rice during the first week of sowing. In 14 days old transplanted rice, both species completely damaged seedlings within two weeks of transplanting. The percentage of missing hills in 21 and 28 days old transplanted rice gradually increased up to 5th and 4th weeks, respectively. After week five, percentages of missing hills recorded in 21 days old transplanted rice caused by *P. maculata* and *P. canaliculata* were $48 \pm 3\%$ and $45 \pm 3\%$, respectively, whereby in

Table 1. Effect of damage of *P. maculata* and *P. canaliculata* to various agronomic and yield components of rice grown by different conventional methods of sowing

	21 Days after transplanting			28 Days after transplanting		
	Control	<i>P. maculata</i>	<i>P. canaliculata</i>	Control	<i>P. maculata</i>	<i>P. canaliculata</i>
No. of tillers	541.2 ± 6.1 ^a	281.6 ± 9.5 ^d	308.0 ± 20.7 ^d	480.0 ± 15.7 ^b	413.6 ± 8.7 ^c	424.0 ± 6.0 ^c
No. of panicles	487.2 ± 5.8 ^a	254.0 ± 9.2 ^d	277.6 ± 18.4 ^d	434.8 ± 14.1 ^b	372.0 ± 7.6 ^c	385.6 ± 5.4 ^c
Leaf Area Index	5.7 ± 0.1 ^a	3.1 ± 0.2 ^c	3.3 ± 0.2 ^c	5.4 ± 0.04 ^{ab}	5.0 ± 0.2 ^b	5.1 ± 0.1 ^b
Spikelets/panicle	126.7 ± 1.7 ^a	128.2 ± 1.2 ^a	127.3 ± 0.7 ^a	122.8 ± 0.6 ^b	126.6 ± 1.0 ^a	125.4 ± 1.0 ^{ab}
Grain filling %	86.4 ± 0.8 ^{ab}	87.0 ± 0.4 ^a	86.9 ± 0.5 ^a	84.1 ± 0.6 ^c	85.4 ± 0.4 ^{bc}	85.3 ± 0.1 ^{bc}
1000 grain weight (g)	27.1 ± 0.03 ^a	27.1 ± 0.02 ^a	27.1 ± 0.02 ^a	27.2 ± 0.01 ^a	27.2 ± 0.03 ^a	27.1 ± 0.1 ^a
Total dry weight (g)	896.6 ± 14.2 ^a	485.2 ± 24.0 ^d	524.4 ± 32.3 ^d	859.8 ± 17.2 ^b	790.6 ± 14.3 ^c	778.4 ± 14.9 ^c
Grain harvest index	50.0 ± 0.3 ^{bc}	50.3 ± 0.2 ^{ab}	50.4 ± 0.4 ^{ab}	49.6 ± 0.3 ^c	50.8 ± 0.1 ^a	50.5 ± 0.1 ^{ab}
Yield (ton/ha)	4.5 ± 0.1 ^a	2.4 ± 0.1 ^c	2.6 ± 0.2 ^c	4.3 ± 0.1 ^{ab}	4.1 ± 0.1 ^b	4.0 ± 0.1 ^b

Note: Means followed by the same letters in the same row are not significantly different ($p < 0.05$).

28 days old transplanted rice, 16±3% and 15±2% missing hills, respectively, were observed after week four. Thereafter, no damage was observed in 21 and 28 days old transplanted rice as snails mostly fed on available rice weeds and detritus material for their nutrition. Many studies also suggested significant role of sowing methods on damage to rice by *Pomacea* spp. (Horgan *et al.*, 2014; Lee *et al.*, 2010; Sanico *et al.*, 2002; Teo, 2003).

Direct seeded rice is the most susceptible to attack of *Pomacea* spp. because of its tender, soft, and succulent parts as compared to transplanted seedlings (Horgan *et al.*, 2014). Accordingly, 100% loss of direct seeded rice by *P. canaliculata* has been reported as compared to 89% and 46% losses in transplanted 21 days and 40 days rice, respectively (Horgan *et al.*, 2014; Teo, 2003). Moreover, reduction in loss of rice seedlings by *Pomacea* spp. has been reported in rice growth as *Pomacea* spp. mostly cause losses to direct seeding rice up to 4 weeks and 2–3 weeks in case of transplanting (Sanico *et al.*, 2002; Teo, 2003).

Agronomic parameters

Considering 100% damage to direct seeding and 14 days transplanted rice, only data for 21 and 28 days transplanted rice were analysed for various agronomic parameters to get the most appropriate mode of cultivation against snails with potential yield.

Results of different agronomic parameters of 21 and 28 days old transplanted rice treatments as given in Table 1 highlighted suggestive differences due to the relatively higher damage by *Pomacea* spp. to 21 days old transplanted rice. The least number of tillers, panicles, and LAI were recorded in snail damaged treatments of 21 and 28 old days old transplanted rice ($p < 0.05$), whereas the respective control treatments showed the highest numbers of these parameters. The highest number of spikelets per panicle was recorded in both snail damaged treatments of 21 days old transplanted rice, but it was not extensively different from the control treatment of 21 days old transplanted rice and 28 days old transplanted rice damaged by snails ($F = 4.22$,

$n=2$, $p > 0.05$). The highest grain filling percentage was recorded in *Pomacea* spp. damaged and control treatments of 21 days old transplanted rice ($F = 20.04$, $n = 1$, $p < 0.05$), whereas no sizeable difference was observed in 1000 grain weight ($F = 0.03$, $n = 2$, $p > 0.05$) in different treatments. The highest yield was recorded in 21 days old transplanted rice control treatment ($F = 42.37$, $n = 2$, $p < 0.001$) with no considerable difference from the yield of 28 days old transplanted rice control treatment. Moreover, yield of all 28 days old transplanted rice treatments was also statistically comparable. Similar to other agronomic parameters, the highest total dry weight was also recorded in 21 days old transplanted rice control treatment followed by 28 days old transplanted rice control and snails damaged treatments ($F = 36.70$, $n = 2$, $p < 0.001$). Although all other agronomic parameters were low in snail infested treatments, all 21 and 28 days old transplanted rice snail damaged treatments showed higher GHI in comparison to their respective control treatments ($F = 6.46$, $n = 2$, $p < 0.05$).

Studies by De Datta (1981) and Naklang *et al.* (1996) emphasized on the adequate age of seedlings for transplanting rice to minimize the transplanting shock and could further result in strong growth of seedlings. Therefore, the use of young seedlings for transplanting minimized the damage to the roots that, in turn, grew vigorously to produce more tillers and panicles, and ultimately higher yield (Krishna *et al.*, 2010; Ranamukhaarachchi, 2011). It is also reported that 14 days old transplanted seedlings produce comparatively more tillers and panicles than 21 and 23 days old seedlings (Makarim *et al.*, 2002). In comparison to the number of tillers, panicles, and LAI, higher number of spikelets per panicle, grain filling percentage, and GHI were recorded in 21 and 28 days old transplanted snail damaged treatments. Accordingly, although higher yields were obtained in 21 and 28 days old transplanted control treatments, yield in all 28 days old transplanted rice was also statistically comparable. The reasons for comparable yields in 21 and 28 days transplanted may be due to the management of adequate spacing between and within rows along with the additional space available due to missing hills in snail introduced treatments. All these factors enable rice plants to optimize the use of soil nutrients, fertilizers, and sunlight to produce higher yields as Awan *et al.* (2011) and Birhane (2013) also highlighted the effect of proper space management in rice for better utilization of nutrients and sunlight along with easy weeding and chemical spray.

Effect of various densities of apple snails to rice grown at two water levels

Percentage damage. The results suggested that different densities of both *Pomacea* spp. did not cause a significant loss to rice cultivated at 2 cm water level and no missing rice hills were observed after the first week of transplanting (Figure 2). However, damage to rice increased with the introduction of water at the 5 cm level ($F = 420.83$, $n = 1$, $p < 0.001$) and more severe losses in shape of missing hills were observed at snail densities of three snails per plot of both species ($F = 33.88$, $n = 2$, $p < 0.001$). Accordingly, level of damage caused by different densities of both species differs significantly at two water levels at weekly intervals ($F = 319.20$, $n = 29$, $p < 0.05$); however, the percentage damage did not differ between two species in the respective treatments ($F = 45.9$,

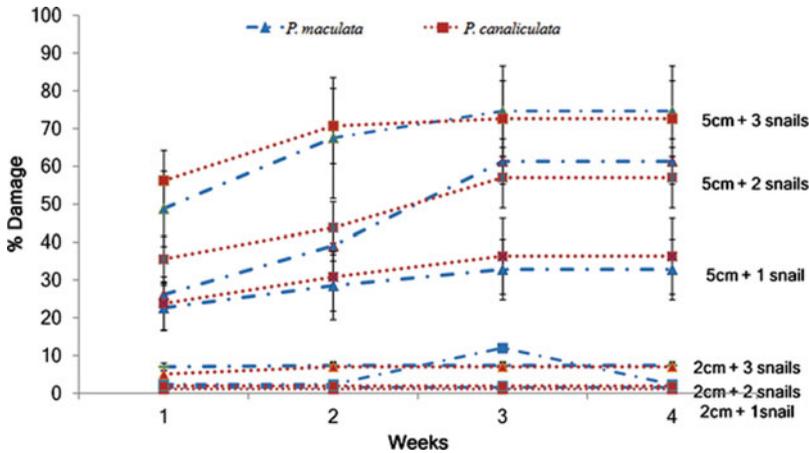


Figure 2. Weekly data on percentage missing hills of rice grown at 2 cm and 5 cm water levels by different densities of *P. maculata* and *P. canaliculata*.

$n = 1$, $p > 0.05$). Previous studies also suggested the significant role of water in the biological activities of *Pomacea* spp. because it is the medium that snails used for their movement (Cowie, 2002; Teo, 2003). Liang *et al.* (2013) while examining the effect of water and duck farming on *P. canaliculata* damage to rice concluded that snails become more active and damaging in flooded rice in comparison to lower or saturated water levels. Zero per cent loss has been reported to crop established through dry direct seeding (Teo, 2003). In this study, at 2 cm water level, three snails per plot density of both species caused maximum damage ($7 \pm 1\%$ missing hills). Results also showed that damage by the two species in 5 cm treatments were recorded up to 3rd week after transplanting. The damage caused by *P. maculata* at densities of 1, 2, and 3 snails per plot was $33 \pm 8\%$, $61 \pm 6\%$, and $75 \pm 12\%$, respectively. The same densities of *P. canaliculata* damaged $36 \pm 10\%$, $57 \pm 8\%$, and $73 \pm 10\%$ seedlings, respectively. Previous studies also confirmed comparatively higher loss of rice seedlings at a snail density of 10 snails per plot as compared to lower snail densities (Lee *et al.*, 2010). Cowie (2002) suggested that level of damage to rice by *Pomacea* spp. is dependent on their size and density as snail density of 8 snails/m² can cause up to 90% loss of young rice seedlings in comparison to 20% loss at 1 snail/m². Studies also confirmed that one adult snail is capable of consuming 24 young seedlings per day and this accordingly signifies its damage to rice (Cowie, 2002). Among the factors responsible for the damage of *Pomacea* spp. to rice, the role of water level is more significant than the stage of rice crop and snail density (Teo, 2003).

Agronomic parameters. Results of the study showed important effect ($p < 0.001$) of water levels and snail densities on various agronomic parameters; however, the difference between *P. maculata* and *P. canaliculata* was trivial in respective treatments (Figure 3). According to the results, although control treatment maintained at 5 cm water level showed maximum agronomic parameters, i.e., number of tillers, number

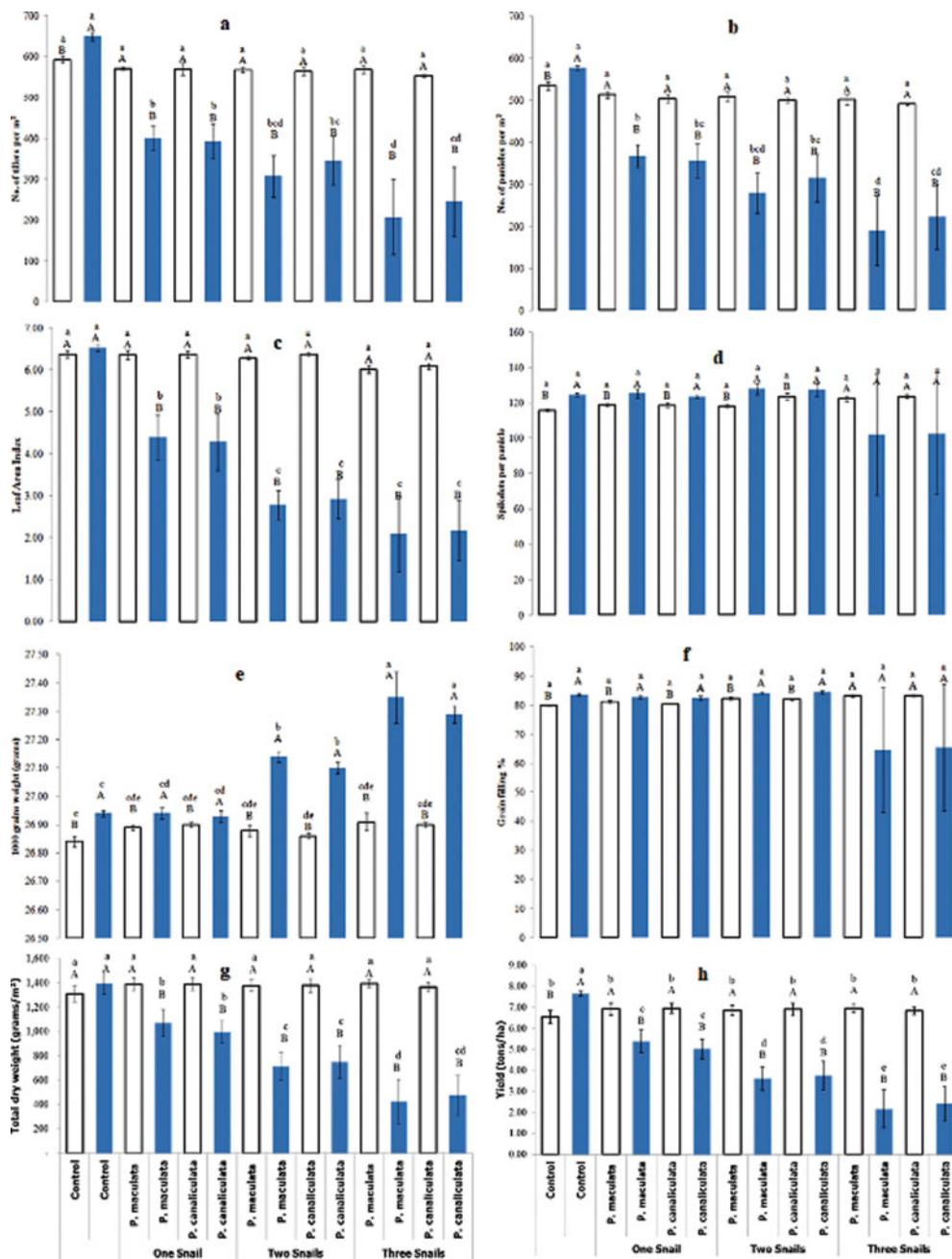


Figure 3. Effect of different water levels and snail densities on various agronomic and yield parameters of rice. *Capital letters indicate significant difference between water levels at the particular snail density ($p < 0.05$). **Small letters indicate significant difference among different snail densities of two *Pomacea* spp. at two water levels ($p < 0.05$). ***White bars = 2cm; Blue bars = 5 cm. a=No. of tillers per m^2 ; b=no. of panicles per m^2 ; c= Leaf Area Index; d=spikelets per panicle; e=1000 grains weight; f=grain filling percentage; g=total dry weight; h=yield.

of panicles and LAI and yield parameters, i.e., spikelets/panicle, grain filling %, 100 grain weight, yield, total dry weight, and GHI; the same were not substantially different from all the treatments maintained at 2 cm water level. This suggested the potential of rice to grow adequately at reduced water levels to produce rice yield comparable with flooded conditions. The potential of alternate practices of rice cultivation, such as alternate wetting and drying, saturated soil culture, ground cover rice, and raised beds for saturated soil culture, has been highlighted to compete with flooded rice for yield with high water saving potential (Peng *et al.*, 2006). However, De Datta (1981), Teo (2003), and Tuong and Bouman (2003) highlighted the significant role of water in early root development and growth of rice plants through exploitation of available nutrients and thus gave higher yields. But it has been suggested that more than 50% irrigation water in rice is lost due to seepage, percolation, and evaporation as the same can be used for more beneficial ventures (Ismail *et al.*, 2013). Results of the study, however, confirmed the significant effect of increasing densities of both *Pomacea* spp. at flooded irrigation ($F = 17.36$, $n = 3$, $p < 0.001$) to cause significant reduction in different agronomic and yield components. Accordingly, the least yield was recorded in rice plots damaged by three snails per plot of both species. Cowie (2002) also reported that yield losses to rice by *P. canaliculata* depends on the density of snails as snail density of eight snails per meter square can reduce the rice yield up to 90% in comparison to 20% yield loss at one snail per meter square.

CONCLUSION

Both *Pomacea* spp. completely damaged direct seeded and 14 days transplanted rice; however, damage to rice decreased with transplanting older seedlings of 21 and 28 days. Higher damage to 28 days old transplanted rice was also recorded at 5 cm water level as compared to 2 cm. No difference in agronomic and yield components was observed between all 2 cm treatments and 5 cm control treatment. Moreover, no significant difference in individual treatments was recorded between two *Pomacea* spp. suggesting their similar damage potential to rice. Therefore, cultivation of rice using 28 days old transplanting seedlings at 2 cm water level is recommended to reduce the damage by *Pomacea* spp. and get comparable yield.

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