# Influence of Azolla on the Survival of Rice Seedlings Planted under Varying Invasive Apple Snail Densities and Water Depths

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## Abstract

*Utilizing azolla to manage invasive apple snail (IAS) has never been reported. Hence, in this study, an experiment was conducted at two trials to determine azolla's influence on the survival of newly transplanted (TPR) and direct-seeded rice (DSR) seedlings planted under varying IAS densities and water depths. Three treatments (T1: rice + IAS, T2: rice + IAS + azolla and T3: rice + IAS + molluscicide), three densities of IAS (5, 10 and 15 snails/0.16 $m^2$) and three water depths (1, 2 and 3 cm) were combined in a three-way factorial arrangement through randomized complete block design with three replications. The number of seedlings that survived and the rate of missing seedlings $day^{-1}$ were measured. Results showed that the TPR's survival at the three water depths ranged from 43 to 65.6% at 2.4 to 4.7 missing seedlings $day^{-1}$ in T1, 84 to 94.8% at 0.5 to 1.4 missing seedlings $day^{-1}$ in T2 and 100% at 0 missing seedlings $day^{-1}$ in T3. No significant differences were observed in the survival of TPR planted in T2 and T3. Meanwhile, the DSR's survival at all water depths and IAS densities ranged from 0 to 3.8% and 0 to 3.6% in T1, 7.4 to 26.8 and 6.5 to 27.2% in T2, respectively, and 100% in T3. DSR had 15.8 missing seedlings $day^{-1}$ in T1 and 13.3 in T2. This study demonstrated that growing azolla could help increase the survival of newly-planted rice, specifically on TPR, against the potential damage of IAS.*

***Keywords**:* biological pest control, invasive apple snail, *Pomacea canaliculata*, golden apple snail, rice pest management

## 1. Introduction

Pests are one of the most important limiting factors in rice production because they can significantly reduce the quantity and quality of harvested rice grains when not controlled. Worldwide estimation from 2001 to 2003 showed that 77 and 37.4% of the potential and actual yields of rice were lost due to pests (Oerke, 2006). Similarly, 37.2% were quantified as yield losses due to pests in tropical Asia (Savary *et al.*, 2000).

Invasive apple snail (*Pomacea canaliculata* [Lamarck]) (IAS), also known as golden apple snail in many pieces of literature, is one of the most important pests of rice in the Philippines (Heong and Escalada, 1997). It was introduced in the country from 1982 to 1984 as a supplement to enrich the protein-deficient diet of consumers and as a new source of livelihood for farmers (Mochida, 1987; Philippine Rice Research Institute [PhilRice], 2003; Cowie *et al.*, 2017). Raisers later abandoned the snails owing to low market demand; hence, they escaped, dispersed and invaded many ricefields. Between 1986 and 1998, IAS infestation expanded rapidly across the country from 300 to 426,000 ha; in 1995, it was more than 800,000 ha (Cagauan and Joshi, 2003). IAS is extremely destructive on newly planted rice seedlings due to their succulent tissues. It is difficult to control once introduced into the field because of its biological and morphological characteristics (Joshi, 2005). Depending on the leaf stages, IAS with a shell height of 5 to 40 mm can attack and significantly damage rice seedlings (Cheng, 1989; Wada, 2006). The snail can cause 8.5 to 16.7%, 41 to 44.2%, 60.5 to 74.2%, 94.7 to 99%, and 100% missing hills on 21-day-old transplanted rice at shell heights of 5, 10, 20, 30 and 40 mm, respectively (Joshi *et al.*, 2002). On direct-seeded rice, the snail can induce 90 to 100% damage at shell height of only 1 to 3 mm (Sin, 2003).

IAS is commonly controlled by way of molluscicide application (Heong and Escalada, 1997; Joshi, 2005; Donayre *et al.*, 2014; Beltran *et al.*, 2016). Many farmers prefer this control method because of ease in application, immediate visibility of the effects and availability in the market. However, the method has been criticized for its several drawbacks, particularly its negative effects on human and animal health, and the environment. Thus, alternative techniques that are effective, economic and safe for the environment have been suggested to control IAS like plant attractants, black carp fish and ducks (Joshi and Dela Cruz, 2001; Adalla and Magsino, 2006; Halwart, 2006; Wada, 2006; Ip, 2013; Amzah and Yahya, 2014).

In the absence of rice seedlings, IAS can divert its diet to other plants and decompose organic matter in the paddies (Mochida, 1991; Carlsson and Lacoursiere, 2005; Carlsson, 2006; Gilal *et al*., 2016; Baloch, 2017). One of these is the azolla (*Azolla* sp.) – a small, free-floating aquatic plant that grows on swamps, ditches, lakes and rivers where the flow of water is not turbulent (Saxena *et al*., 1987; Khan, 1988; Quebral, 1989; Carlsson, 2017). Azolla can fix atmospheric nitrogen through a symbiotic relationship with the filamentous nitrogen-fixing bacterium *Anabaena azollae* (Peters and Meeks, 1989). Thus, it is a popular plant used and recommended as an alternative source of nutrients for rice and feed supplements for the animals and fishes on the farm (Lales *et al*., 1989; Querubin *et al*., 1989a, 1989b; Sevilla *et al*., 1989; Heuze and Tan, 2017). It has been also found as an effective control against certain weeds of rice like *Echinochloa crus-galli* (L.) P. Beauv., *Echinochloa glabrescens* Munro ex Hook. f., *Cyperus difformis* L., *Aechynomene indica* L., *Eclipta prostrata* L., *Monochoria vaginalis* (Burm. f.) C. Presl. (Satapathy and Singh, 1985; Krock *et al*., 1991; Nakornsri *et al*., 1999; Biswas *et al*., 2005; Shin *et al*., 2021).

The use of attractants has also been considered an alternative way of controlling IAS. For example, Amzah and Yahya (2014) reported that jackfruit (*Artocarpus heterophyllus* Lam.) effectively lured the snail by 28.8% compared with other attractants like cassava leaves (*Manihot esculenta* Crantz), water spinach (*Ipomoea aquatica* Forsk.) and banana leaves (*Musa* sp.). Joshi and de la Cruz (2001) and Teo (2004) also revealed that leaves of taro (*Colocasia esculenta* [L.] Schott), papaya (*Carica papaya* L.), sweet potato (*Ipomoea batatas* [L.] Lam.), cassava and gliricidia (*Gliricidia sepium* [Jacq.] Kunth ex Walp.) were effective attractant to IAS. On the other hand, a laboratory experiment showed that *Azolla filiculoides* Lam. was preferred by IAS over *A. pinnata* R. Br., *A. rubra* R. Br., *A. Mexicana* Presl., *A. microphylla* Kaulf. and *A. caroliniana* Willd. (Cagauan, 2002). Results of the experiment suggested that azolla could be a potential biological attractant to IAS, facilitating the collection of the snail in the field. Despite the significant findings, the effects of azolla on the survival of rice in relation to the potential damage by IAS were not considered. Thus, this study was conducted to determine the influence of azolla on the survival of newly transplanted (TPR) and direct-seeded rice (DSR) seedlings planted under varying invasive apple snail (IAS) densities and water depths.

## 2. Methodology

### *2.1 Time and Place of Experiment*

The study was conducted in two trials at the experimental area of the PhilRice Negros Station (PhilRice Negros), Cansilayan, Murcia, Negros Occidental, Philippines (10° 34’ 1.93” N, 122° 59’ 36.43” E) from November 2014 to February 2015.

### *2.2 Culture Medium and Rice Seedlings Preparation*

The culture medium for growing rice plants was prepared by collecting composite soil samples from the organic rice area of PhilRice Negros. Collected soil was air-dried, pulverized, sieved and sterilized for 2 h at 100 °C. After sterilization, 10 kg of pre-cooled composite soil was transferred into a plastic container measuring 40 x 40 x 20 cm (L x W x H). A total of 2 kg of organic fertilizer and 5-L clean tap water were then simultaneously added and mixed with the soil to complete the contents of the culture medium. To determine the physicochemical characteristics of the culture medium, 1 kg each of the composite soil and the organic fertilizer was sent to the Philippine Sugar Research Institute Foundation, Inc., Victoria Experiment Station, Victorias City, Negros Occidental for analysis. The results of the analysis are shown in Table 1. Meanwhile, test plants for transplanting and direct-seeding were prepared following the standard procedure of PhilRice (2007).

Table 1. Physicochemical characteristics of culture medium

| Physicochemical characteristics | Composite soil | Organic fertilizer (vermicast) |
|---|---|---|
| Nitrogen (%) | 0.08 | 1.55 |
| Phosphorus (%) | 0.001 | 0 |
| Potassium (%) | 0.012 | 0.04 |
| Calcium (%) | 0.043 | 3.63 |
| Magnesium (%) | 0.008 | 0.22 |
| Sodium (%) | - | 0.03 |
| Zinc (%) | - | 0.02 |
| Iron (%) | - | 0.53 |
| Copper (%) | - | 0.01 |
| Sulfur (%) | - | 0.09 |
| pH | 5.19 | 5.29 |
| Organic matter (%) | 1.03 | 24.21 |
| Moisture content (%) | - | 36.6 |

### *2.3 Collection and Mass Production of Azolla*

Azolla plants (*Azolla* sp.) growing at the organic rice production area of PhilRice Negros were collected, transferred to black plastic containers and brought to the experimental area of the station for mass production and maintenance in the culture medium. A total of 100 g of azolla plants were transferred to the culture medium to further enhance growth and development. The morphological characteristics of *Azolla* sp. used in this experiment resembled those *A. microphylla*: circular or fan-shaped outline, light green and spore-forming (Tan *et al*., 1986).

### *2.4 Collection and Maintenance of Invasive Apple Snails*

IAS individuals were collected at the seedling nursery and brought into the experimental area of PhilRice Negros for measurement using a digital caliper (0-150 mm, Mitutoyo, Japan). Snail individuals with shell heights of 20 to 30 mm were selected, washed and starved for 24 h inside a 20-L plastic container filled with 15-L tap water. To prevent IAS from escaping, each bucket was covered with a fine, nylon net and later kept inside the room under cool conditions (25 °C; 85% relative humidity).

### *2.5 Experimental Treatments and Design*

Plastic containers, measuring 40 x 40 x 20 cm and containing culture medium, were used as the experimental units of this study. Three treatments (T1: rice + IAS, T2: rice + IAS + azolla and T3: rice + IAS + molluscicide (metaldehyde: 0.47 g/L)], three densities of IAS (5, 10, and 15 snails/0.16 $m^2$ derived from 30, 60 and 90 snails/$m^2$) and three water depths (1, 2, and 3 cm) were combined in a three-way factorial experiment arranged in a randomized complete block design with three replications.

Twenty-one-day-old seedlings of NSIC Rc 222 were transplanted into the experimental unit at a distance of 20 x 20 cm (row x hill). For the direct seeding method, three-day-old pre-germinated seeds were seeded equidistantly at 4 x 4 cm. The levels of water in each experimental unit for both establishment methods were kept saturated (0 cm above the soil) within 10 days to enhance good growth and development of seedlings. After 10 days, azolla at 90 g/0.16 $m^2$ (enough to cover the whole medium) was applied and evenly distributed in each experimental unit. Simultaneously, water and IAS at different levels were also applied.

All units were then covered with fine nets to prevent the snails from escaping. The level of water in each unit was monitored twice a day by measuring with a ruler. Water was added where it dropped below the desired level. The number of seedlings that survived in each experimental unit was observed and recorded within seven days after planting. At the end of the experiment, the percentage of seedlings that survived (SS) and the rate of missing seedlings day$^{-1}$ (RMS) were calculated using Formulae 1 and 2, respectively.

$$SS\ (\%) = \left(\frac{B}{A}\right) * 100 \qquad (1)$$

where *B* is the total number of seedlings that survived and *A* is the total number of seedlings planted.

$$RMS = \left(\frac{Y}{Xa}\right) + \ldots + \left(\frac{Y}{Xg}\right) \qquad (2)$$

where *Y* is the total number of missing seedlings, *Xa* is the day of first count and *Xg* is the day of final count.

*2.6 Statistical Analysis*

Data on the percentage of survival and rate of missing seedlings were subjected to analysis of variance (ANOVA) using the Statistical Tool for Agricultural Research of the International Rice Research Institute (version 2014). Since prior analyses showed no significant interactions between trials and treatments, all data of the two trials in each establishment method were pooled and analyzed in a three-way ANOVA. Prior analyses also showed variance heterogeneity of data. Thus, all means were transformed using the square root transformation method $(X + 0.5)^{1/2}$ (Gomez and Gomez, 1984). All transformed means were then compared using Fisher's least significant difference (LSD) at a 5% level of significance.

## 3. Results and Discussion

Three-way ANOVA showed that the main effects of treatments (Ts) and water depths (WDs) were both significant on the survival and rate of missing seedlings day$^{-1}$ of newly transplanted rice (Table 2). Snail densities (SPs) were only significant on the rate of missing seedlings day$^{-1}$. The interaction effect of Ts x WDs was significant on both survival and the rate of missing seedlings day$^{-1}$. However, the interaction effects of SPs x WDs and Ts x SPs x WDs were not significant on survival. On direct-seeded rice, the main effect of Ts was significant on both survival and rate of missing seedlings day$^{-1}$ while the main effect of SPs and WDs was only on survival. The interaction effects of Ts x SPs and Ts x WDs were significant only on survival. Also, the interaction effects of SPs x WDs and Ts x SPs x WDs were not significant on survival. Likewise, the main effects of SPs and WDs were not significant on the rate of missing seedlings day$^{-1}$; the same trends were observed in Ts x SPs, Ts x WDs, SPs x WDs and Ts x SPs x WDs.

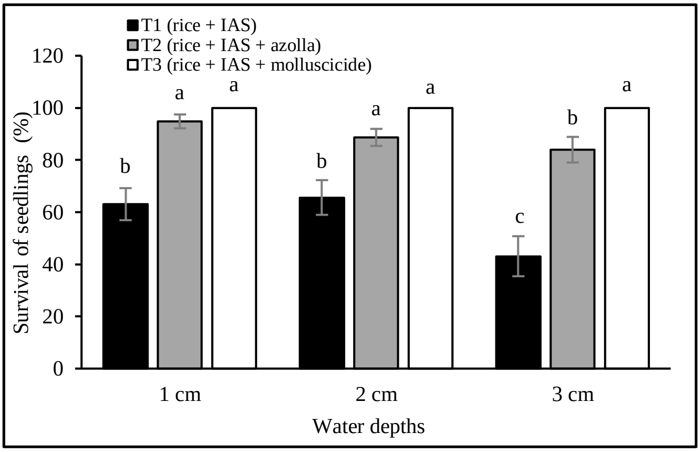
Table 2. P-values for the effects of treatments, snail densities and water depths on percentage of survival and rate of missing hills of newly planted rice

| Factors | Transplanted rice (TPR) | | Direct-seeded rice (DSR) | |
|---|---|---|---|---|
| | Survival (%) | Missing seedlings day$^{-1}$ | Survival (%) | Missing seedlings day$^{-1}$ |
| Treatments (Ts) | .000$^{**}$ | .000$^{**}$ | .000$^{**}$ | .000$^{**}$ |
| Snail densities (SPs) | .055$^{ns}$ | .020$^{*}$ | .000$^{**}$ | .056$^{ns}$ |
| Water depths (WDs) | .001$^{**}$ | .016$^{*}$ | .000$^{**}$ | .065$^{ns}$ |
| Ts x SPs | .214$^{ns}$ | .119$^{ns}$ | .000$^{**}$ | .051$^{ns}$ |
| Ts x WDs | .003$^{**}$ | .037$^{*}$ | .000$^{**}$ | .054$^{ns}$ |
| SPs x WDs | .623$^{ns}$ | .785$^{ns}$ | .194$^{ns}$ | .119$^{ns}$ |
| Ts x SPs x WDs | .699$^{ns}$ | .803$^{ns}$ | .256$^{ns}$ | .064$^{ns}$ |

$^{*}p < .05$, $^{**}p < .005$, and ns – not significant at 5% level of significance

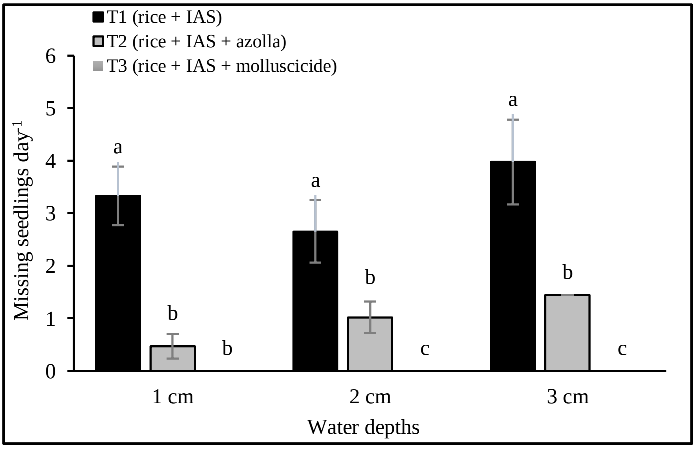
Regardless of water depths, the percentage survival of newly-transplanted rice was highest in T3 (all 100%) followed by T2 (1 cm = 94.8, 2 cm = 88.7 and 3 cm = 84%) as shown in Figure 1. No significant differences were noted in the percentage of survival of TPR applied with molluscicide and azolla except at 3 cm water depth. T1 had the least percentage of survival of seedlings at three water depths (1 cm = 63.1, 2 cm = 65.6 and 3 cm = 43%). The percentages of survival of newly-transplanted rice in T1 and T2 decreased as water depth increased. Nevertheless, the survival of transplanted rice was still highest in T2 as compared to T1. In addition, the rate of missing seedlings per day$^{-1}$ (due to feeding of IAS) in T2 was lower at all water depths (1 cm = 0.5, 2 cm = 1, and 3 cm = 1.4 seedlings day$^{-1}$) than in T1 (1 cm = 3.3, 2 cm = 2.4 and 3 cm = 4.7 seedlings day$^{-1}$) (Figure 2).

The percentage of survival of DSR was also highest (100%) in T3 regardless of water depths (Figure 3) and IAS densities (Figure 4). However, lower percentages of survival of seedlings were observed in T2 at different water depths (1 cm = 26.8, 2 cm = 17 and 3 cm = 7.4%) and IAS densities (1 cm = 27.2, 2 cm = 17.5 and 3 cm = 6.5%). The percentage of survival decreased as water depth increased. T1 had the very least survival of seedlings at all water depths (0-3.8%) and IAS densities (0-3.6%). Despite that, the rate of missing seedlings of DSR was lower in T2 (13.3 seedlings day$^{-1}$) than in T1 (15.8 seedlings day$^{-1}$) as shown in Figure 5.



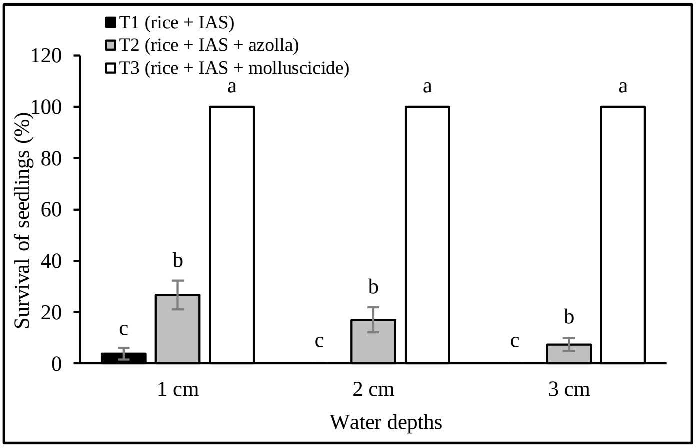

Means with the same letter are not significantly different at 5% level of significance using LSD; error bars are + SE of the means.

Figure 1. Survival of newly transplanted rice as influenced by different treatments and water depths



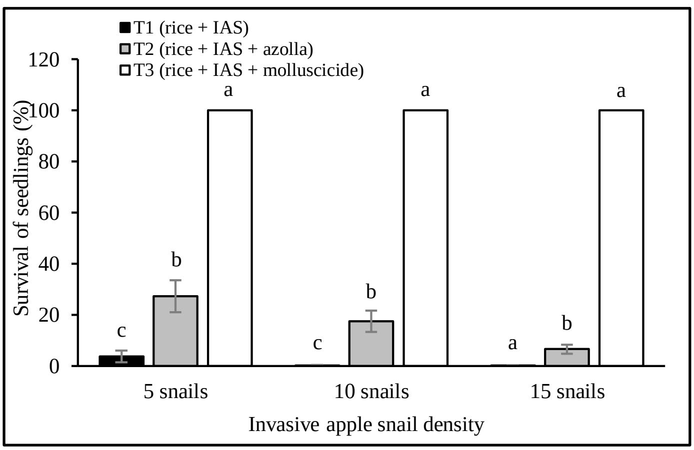

Means with the same letter are not significantly different at 5% level of significance using LSD; error bars are + SE of the means.

Figure 2. Rate of missing seedlings of newly transplanted rice as influenced by different treatments and water depths




Means with the same letter are not significantly different at 5% level of significance using LSD; error bars are + SE of the means.

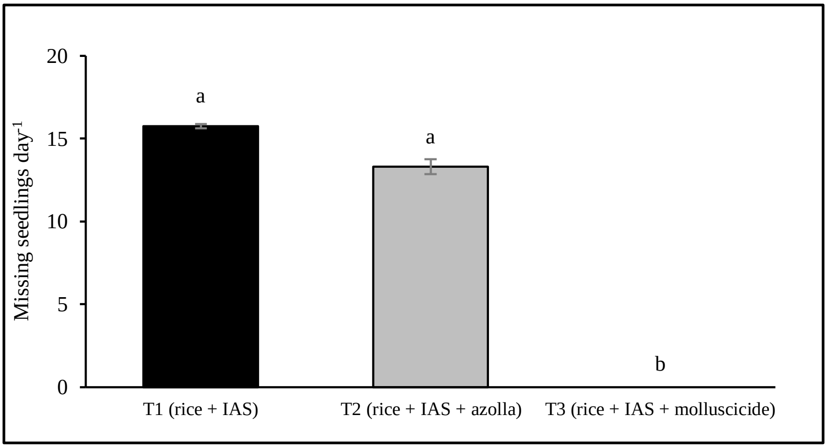
Figure 3. Survival of newly seeded rice as influenced by different treatments and water depths




Means with the same letter are not significantly different at 5% level of significance using LSD; error bars are + SE of the means.

Figure 4. Survival of newly seeded rice as influenced by different treatments and invasive apple snail densities

This study demonstrated that growing azolla could also help increase the survival and reduce the rate of missing seedlings of newly-planted rice despite the proliferation of IAS having shell heights of 20 to 30 mm. The results specified, however, that azolla had more positive effects on the survival of TPR than DSR. This was probably due to the advantage of TPR in terms of age, which complemented the influence of azolla on the survival of seedlings against the damaging activity of IAS. Sanico *et al.* (2002) reported that transplanting two, three, four and five-week-old rice seedlings at 8, 6, 4 and 2 seedlings hill$^{-1}$, respectively, significantly reduced the percentage of missing hills by 0 to 0.2 % in the dry season and 1.4 to 11% in the wet season. The researchers added that transplanting older seedlings resulted in minimal grain yield losses. Sin (2003) also revealed that transplanting 30- and 40-day-old rice seedlings resulted in only 8.6 and 0% damage by IAS at 5 cm water depth.




Means with the same letter are not significantly different at 5% level of significance using LSD; error bars are + SE of the means.

Figure 5. Rate of missing seedlings of newly seeded rice as influenced by different treatments

The influence of azolla on the survival of rice under high densities of IAS decreased as water depth was increased. This was probably due to less accessibility of snails to azolla at deeper water levels. When azolla floated further away, what was left under the water were the succulent body parts of the newly planted rice that had been easily grazed and damaged by the snails. In a study on the effect of snail density and water depths on missing seedlings of 28-day old TPR caused by two *Pomacea* snails, Arfan *et al.* (2016) reported that one, two and three snails plot$^{-1}$ (2 x 2 m) did significantly reduced the missing seedlings at 2 cm water depth. However, at 5 cm depth, the percentage of missing hills became 33, 61 and 75% as damaged by *P. maculata* at one, two and three snails plot$^{-1}$; 36, 57 and 73% as affected by *P. canaliculata* in the same order of density, respectively.

Previous researchers showed that DSR had a high vulnerability to the damage potential of IAS. For example, Sin (2003) reported that 90 to 100% of DSR were damaged by snails with shell heights of 1 to 3 mm. The author added that 100% of DSR were damaged when the densities of snails was set at 1, 2, 3, 4 and 5 $m^{-2}$ at water depths of 1, 2.5, 5 and 10 cm. Wada *et al*. (1999) also found that 100% of DSR was damaged by snails when water was drained one day after seeding. The present study also obtained the same results as the previous works: less number of seedlings survived in direct-seeded rice. However, it was discovered that newly seeded rice added with azolla had a higher percentage of survival than those that had none.

## 4. Conclusion and Recommendation

Azolla had an influence on the survival and missing seedlings of rice under varying densities of invasive apple snail, particularly on transplanted rice. However, its influence decreased as water depth increased. The findings could be used as basis for the development of pest management against IAS.

The present study was conducted in small plastic containers and may not all represent the field situations where many factors could affect efficacy of azolla as attractant to IAS. To further validate the study's conclusions, it is recommended that more experiments be conducted particularly under field conditions. Future works should also integrate other techniques such as good land preparation, planting of older seedlings at optimum numbers $hill^{-1}$, water depth management and herding of ducks at specific periods of rice to come up with a holistic apple snail management strategy that is economical, sustainable and environment-friendly under Philippine conditions.

## 5. Acknowledgement

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## 6. References

Adalla, C.B., & Magsino, E.A. (2006). Understanding the golden apple snail *(Pomacea canaliculata*): Biology and early initiatives to control the pest in the Philippines. In: R.C. Joshi & L.S. Sebastian (Eds.), Global advances in ecology and management of golden apple snail (pp. 199-213). Science City of Muñoz, Nueva Ecija, Philippines: Philippine Rice Research Institute.

Amzah, B., & Yaahya, H. (2014). Evaluation of several plant-based attractants for apple snail management. Acta Biologica Malaysiana, 3(2), 49-57. http://dx.doi.org/10.7593/abm/3.2.49

Arfan, A.G., Muhamad, R., Omar, D., Nor Azwady, A.A., & Manjeri, G. (2016). Sowing methods and water levels influence apple snail damage to rice and its yield in peninsular Malaysia. Experimental Agriculture, 53(4), 1-11. https://doi.org/10.1017/S0014479716000557

Baloch, W.A. (2017). Country report (Pakistan) on non-native apple snails. In: R.C. Joshi, R.H. Cowie & L.S. Sebastian (Eds.), Biology and management of invasive apple snails (pp. 213-220). Science City of Muñoz, Nueva Ecija, Philippines: Philippine Rice Research Institute.

Beltran, J.C., Bordey, F.H., Launio, C.C., Litonjua, A.C., Manalili, R.G., Mataia, A.B., Relado, R.Z., & Moya, P.F. (2016). Pesticide use and practices. In: F.H. Bordey, P.F. Moya, J.C. Beltran & D.C. Dawe (Eds.), Competitiveness of Philippine rice in Asia (pp. 75-86). Science City of Muñoz, Nueva Ecija, Philippines: Philippine Rice Research Institute.

Biswas, M., Parveen, S., Shimozawa, H., & Nakagoshi, N. (2005). Effects of azolla species on weed emergence in a rice (*Oryza sativa*) paddy ecosystem. Weed Biology and Management, 5(4), 176-183. https://doi.org/10.1111/j.1445-6664.2005.00177.x

Cheng, E.Y. (1989). Control strategy for the introduced snail *Pomacea lineata* in rice paddy. British Crop Protection Council Monograph, 41, 69-75.

Cagauan, A.G. (2002). Appetency test for different azolla species to adult golden apple snail *Pomacea canaliculata* Lam. in laboratory condition. Retrieved from http://agris.fao.org/agris-search/search.do?recordID=PH2003001105

Cagauan, A.G., & Joshi, R.C. (2003). Golden apple snail *Pomacea* spp. in the Philippines: Review on levels of infestation, control methods, utilization and future directions. Journal Agricultural Life and Sciences, 37, 7-32.

Carlsson, N.O.L. (2006). Invasive golden apple snails are threatening natural ecosystems in Southeast Asia. In: R.C. Joshi & L.S. Sebastian (Eds.), Global advances in ecology and management of golden apple snail (pp. 61-72). Science City of Muñoz, Nueva Ecija, Philippines: Philippine Rice Research Institute.

Carlsson, N.O.L. (2017). Invasive golden apple snails are threatening natural ecosystems in Southeast Asia, Europe, and North America. In: R.C. Joshi, R.H. Cowie

& L.S. Sebastian (Eds.), Biology and management of invasive apple snails (pp. 45-62). Science City of Muñoz, Nueva Ecija, Philippines: Philippine Rice Research Institute.

Carlsson, N.O.L., & Lacoursiere, J.O. (2005). Herbivory on aquatic vascular plants by the introduced golden apple snail (*Pomacea canaliculata*) in Lao PDR. Biological Invasions, 7, 233-241. https://doi.org/10.1007/s10530-004-0741-4

Cowie, R.H., Hayes, K.A., Strong, E.E., & Thiengo, S.C. (2017). Non-native apple snails: systematics, distribution, invasion history, and reasons for introduction. In: R.C. Joshi, R.H. Cowie & L.S. Sebastian (Eds.), Biology and management of invasive apple snails (pp. 3-32). Science City of Muñoz, Nueva Ecija, Philippines: Philippine Rice Research Institute.

Donayre, D.K.M., Endino, C.A., Seville, C.U., & Ciocon, I.M.G. (2014). Predominant pests and pest management practices of rainfed rice farmers in Negros Island, Philippines. Asia Life Sciences, 23(2), 497-514.

Gilal, A.A., Muhamad, R., Omar, D., Aziz, N.A.A, & Gnanasegaram, M. (2016). 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.. Pakistan Journal of Zoology, 48(3), 673-679.

Gomez, K.A., & Gomez, A.A. (1984). Statistical procedures for agricultural research. United States: John Wiley & Sons.

Halwart, M. (2006). Fish as biological control agents of golden apple snails in Philippine rice fields. In: R.C. Joshi & L.S. Sebastian (Eds.), global advances in ecology and management of golden apple snail (pp. 364-374). Science City of Muñoz, Nueva Ecija, Philippines: Philippine Rice Research Institute.

Heong, K.L., & Escalada, M.M. (1997). A comparative analysis of pest management practices of rice farmers in Asia. In: K.L. Heong & M.M. Escalada (Eds.), Pest management of rice farmers in Asia (pp. 369-386). College, Laguna, Philippines: International Rice Research Institute.

Heuze, V., & Tan, G. (2017). Apple snails as animal feed. In: R.C. Joshi, R.H. Cowie & L.S. Sebastian (Eds.), Biology and management of invasive apple snails (pp. 3-32). Science City of Muñoz, Nueva Ecija, Philippines: Philippine Rice Research Institute.

Ip, K.K.L. (2013). Biological control of golden apple snails (*Pomacea canaliculata*) in freshwater wetland using black carp (*Mylopharyngodon piceus*) (Dissertation). Hong Kong Baptist University, Hong Kong.

Joshi, R.C., Dela Cruz, M.S., Martin, A.R., Duca, A.V., & Martin, E.C. (2002). Relation of golden apple snail size to rice seedling damage in transplanted and direct-seeded rice cultivation. International Rice Research Notes, 27(1), 37-38.

Joshi, R.C. (2005). Managing invasive alien mollusc species in rice. International Rice Research Notes, 30(2), 5-13.

Joshi, R.C. & Dela Cruz, M.S. (2001). Newspaper: A new attractant for golden apple snail management. International Rice Research Notes, 26(2), 49-50.

Khan, M.M. (1988). A primer on azolla production and utilization in agriculture. Laguna, Philippines: University of the Philippines Los Baños.

Krock, T., Alkamper, J., & Watanabe, I. (1991). Azolla contribution to weed control in rice cultivation. Plant Research and Development, 34, 117-125.

Lales, J.S., Lapitan, M.A., & Marte, R.S. (1989). Azolla-rice-fish culture. Azolla: Its culture, management and utilization in the Philippines (pp. 167-180). Laguna, Philippines: University of the Philippines Los Baños.

Mochida, O. (1987). *Pomacea* snails in the Philippines. International Rice Research Newsletters 12(4), 48-49.

Mochida, O. (1991). Spread of freshwater *Pomacea* snails (pilidae, mollusca) from Argentina to Asia. Micronesia Supplementum, 3, 51-62.

Nakornsri, K., Vongsaroj, P., & Inthalang, S. (1999). Rate of azolla (*Azolla pinnata* B.Br.) on weed control in pre-germinated direct-seeded rice. Thai Agricultural Research Journal, 17(3), 303-309.

Oerke, E.C. (2006). Crop losses to pests. Journal of Agricultural Science, 144, 31-43. https://doi.org/10.1017/S0021859605005708

Peters, G.A., & Meeks, J.C. (1989). The azolla-anabaena symbiosis: Basic biology. Annual Review of Plant Physiology and Plant Molecular Biology, 40, 193-210. https://doi.org/10.1146/annurev.pp.40.060189.001205

Philippine Rice Research Institute (PhilRice). (2003). Management options for the golden apple snail. Science City of Muñoz, Nueva Ecija, Philippines: Philippine Rice Research Institute.

Philippine Rice Research Institute (PhilRice). (2007). Philippine rice production training manual. Science City of Muñoz, Nueva Ecija, Philippines: Philippine Rice Research Institute.

Quebral, N.C. (1989). Introduction to azolla and its uses. Azolla: Its culture, management and utilization in the Philippines (pp. 5-22). Laguna, Philippines: University of the Philippines Los Baños.

Querubin, L.J., Alcantara, P.F., & Princes, A.O. (1989a). Chemical composition of three azolla species (*A. caroliniana*, *A. microphylla*, and *A. pinnata*) and feeding vale of azolla meal (*A. microphylla*) in broiler ration II. Azolla: Its culture, management and utilization in the Philippines (pp. 181-194). Laguna, Philippines: University of the Philippines Los Baños.

Querubin, L.J., Alcantara, P.F., Luis, E.S., Princes, A.O., & Paiaspas, V.O. (1989b). Azolla (*A. microphylla*) silage for growing pigs. Azolla: Its culture, management and utilization in the Philippines (pp. 195-214). Laguna, Philippines: University of the Philippines Los Baños.

Sanico, A.L., Peng, S., Laza, R.C., & Visperas, R.M. (2002). Effect of seedling age and seedling number per hill of snail damage in irrigated rice. Crop Protection, 21, 137-143. https://doi.org/10.1016/S0261-2194(01)00075-8

Satapathy, K.B., & Singh, P.K. (1985). Control of weeds by azolla in rice. Journal of Aquatic Plant Management, 23, 40-42.

Savary, S., Willocquet, L., Elazegui, F.A., Castilla, N.P., & Teng, P.S. (2000), Rice pest constraints in tropical Asia: Quantification of yield losses due to rice pests in a range of production situations. Plant Disease, 84, 357-369. https://doi.org/10.1094/PDIS.2000.84.3.357

Saxena, R.C., De Lara, A.V., & Justo, H.D., Jr. (1987). Golden apple snail: A pest of rice. International Rice Research Newsletter, 12(1), 24.

Sevilla, C.C., Perez, C.B., Jr., Batungbacal, M.R., & Natural, H.G., Jr. (1989). Azolla as feed for goats. Azolla: Its culture, management and utilization in the Philippines (pp. 215-220). Laguna, Philippines: University of the Philippines Los Baños.

Sin, T.S. (2003). Damage potential of the golden apple snail *Pomacea canaliculata* (Lamarck) in irrigated rice and its control by cultural approaches. International Journal of Pest Management, 49(1), 49-55. https://doi.org/10.1080/713867835

Shin, J.J., Han, C.M., Kwon, J.B., & Won, J.G. (2021). Effects of azolla incorporation during paddy cultivation on weed control, nitrogen supply, and rice yield. The Korean Journal of Crop Science, 66(4), 271-278.

Teo, S.S. (2004). Integrated control of the golden apple snail (*Pomacea canaliculata* Lamarck, 1822) in rice. Proceedings of the APEC Symposium on the Management of the Golden Apple Snail, Ping Tung, Taiwan, ROC.

Wada, T. (2006). Impact and control of introduced apple snail, *Pomacea canaliculata* (Lamarck), in Japan. In: R.C. Joshi & L.S. Sebastian (Eds.), Global advances in ecology and management of golden apple snail (pp. 181-197). Science City of Muñoz, Nueva Ecija, Philippines: Philippine Rice Research Institute.

Wada, T., Ichinose, K., & Higuci, H. (1999). Effect of drainage on damage to direct-sown rice by the apple snail *Pomacea canaliculata* (Lamarck) (Gastropoda: Ampullariidae). Applied Entomology and Zoology, 34(3), 365-370. https://doi.org/10.1303/aez.34.365

Tan, R.C., Payawal, P., Watanabe, I., Lacdan, N., & Rami, C. (1986). Modern taxonomy of azolla: A review. Philippine Agriculturist, 69, 491-512.