Sains Malaysiana 52(1)(2023): 71-81
http://doi.org/10.17576/jsm-2023-5201-06

The Potential of Several Wild Invasive Fish Species as Fish-Based Organic Fertilizers on the Growth of Two Common Vegetables in Malaysia

(Potensi Beberapa Spesies Ikan Invasif Liar sebagai Baja Organik Berasaskan Ikan ke atas Pertumbuhan Dua Sayuran Umum di Malaysia)

Jennifer Anak Fabian Unggang, Mohd. Nizam Bakar & Ahmad Bukhary Ahmad Khair*

School of Biological Sciences, G08 Building, Universiti Sains Malaysia, 11800 USM Penang, Malaysia

Received: 28 June 2021/Accepted: 21 April 2022

ABSTRACT

Three common invasive fish species in Malaysia, Peacock Bass (*Cichla ocellaris*), Nile Tilapia (*Oreochromis niloticus*) and Algae Suckermouth Catfish (*Hypostomus plecostomus*) were assessed for their efficacy as potential fish powder fertilizers. These invasive fish species were known to disturb the stability of lentic and lotic aquatic ecosystems in Malaysia, especially *O. niloticus* and *H. plecostomus*, altering aquatic habitats and food webs to be unsuitable for survival of indigenous fish species, while *C. ocellaris* becoming active aggressive predators on indigenous fish species, overall reducing indigenous fish species diversity. Plant primary macronutrient traces showed that *C. ocellaris* fish powder fertilizer recorded the highest Nitrogen (N) element percentage (15.81 ± 0.43 N %w/w) and trace Potassium (K) element (28,909.15 ± 32.56 K mg/kg), while *H. plecostomus* fish powder fertilizer recorded the highest trace Phosphorus (P) element (30,562.09 ± 197.11 P mg/kg). Plant secondary macronutrient traces showed that *C. ocellaris* fish powder fertilizer recorded the highest trace Magnesium (Mg) element (1496.66 ± 3.99 Mg mg/kg), while *H. plecostomus* fish powder fertilizer recorded the highest trace Calcium (Ca) element (6984.48 ± 26.20 Ca mg/kg). Two vegetable species tested for their growths, the water spinach (*Ipomoea aquatica*) and the spinach (*Spinacia oleracea*), showed that *C. ocellaris* fish powder fertilizer recorded the highest heights (263.74 ± 12.29 mm, 166.35 ± 9.46 mm), the widest leaf width (14.82 ± 0.66 mm, 21.08 ± 1.53 mm), and the widest stalk width (3.06 ± 0.10 mm, 2.89 ± 0.17 mm), respectively, comparable to the NPK 15:15:15 compound fertilizer, followed by *H. plecostomus* and *O. niloticus* fish powder fertilizers. *C. ocellaris* as predatory invasive fish species with the highest Mg concentration and moderate Ca concentrations, was especially suitable for the growth of both semi-aquatic and terrestrial vegetables grown on low Mg concentration soils.

Keywords: *Cichla ocellaris*; *Hypostomus plecostomus*; *Oreochromis niloticus*; organic fertilizer; vegetables

ABSTRAK

Tiga spesies ikan invasif umum di Malaysia, Ikan Raja (*Cichla ocellaris*), Tilapia Nil (*Oreochromis niloticus*) dan Ikan Bandaraya (*Hypostomus plecostomus*) dinilai terhadap keberkesanannya sebagai baja serbuk ikan yang berpotensi. Ikan invasif ini diketahui mengganggu kestabilan ekosistem akuatik lentik dan lotik di Malaysia, lebih-lebih lagi *O. niloticus* dan *H. plecostomus*, mengubah habitat akuatik dan siratan makanan menjadi tidak sesuai kepada kemandirian spesies ikan asli, manakala *C. ocellaris* menjadi pemangsa aktif agresif ke atas spesies ikan asli, secara keseluruhannya menurunkan kepelbagaian spesies ikan asli. Surihan makronutrien tumbuhan primer menunjukkan baja serbuk ikan *C. ocellaris* merekodkan peratusan unsur Nitrogen (N) (15.81 ± 0.43 N %w/w) dan surihan unsur Kalium (K) (28,909.15 ± 32.56 K mg/kg) tertinggi, manakala baja serbuk ikan *H. plecostomus* merekodkan surihan unsur Fosforus (P) tertinggi (30,562.09 ± 197.11 P mg/kg). Surihan makronutrien tumbuhan sekunder menunjukkan baja serbuk ikan *C. ocellaris* merekodkan surihan unsur Magnesium (Mg) tertinggi (1496.66 ± 3.99 Mg mg/kg), manakala baja serbuk ikan *H. plecostomus* merekodkan surihan unsur Kalsium (Ca) tertinggi (6984.48 ± 26.20 Ca mg/kg). Dua spesies sayuran yang diuji dari segi pertumbuhan iaitu kangkung (*Ipomoea aquatica*) dan bayam (*Spinacia oleracea*), masing-masing menunjukkan bahawa baja serbuk ikan *C. ocellaris* merekodkan ketinggian tertinggi (263.74 ± 12.2 mm; 166.35 ± 9.46 mm), lebar daun yang paling besar (14.82 ± 0.66 mm; 21.08 ± 1.53 mm) dan batang yang paling tebal (3.06 ± 0.10 mm; 2.89 ± 0.17 mm), sebanding dengan baja sebatian NPK 15:15:15, diikuti oleh baja serbuk ikan *H. plecostomus* dan *O. niloticus*. *C. ocellaris* sebagai ikan invasif pemangsa dengan kepekatan tertinggi Mg dan kepekatan Ca sederhana, sesuai untuk pertumbuhan sayuran separa akuatik dan daratan yang ditanam pada tanah yang rendah kepekatan Mg.

Kata kunci: Baja organik; *Cichla ocellaris*; *Hypostomus plecostomus*; *Oreochromis niloticus*; sayuran

Introduction

Biodiversity of the global and national freshwater ecosystem is declining at an alarming rate, with the already existing intentional and unintentional anthropogenic disturbances coupled with the harmful impacts of invasive alien fish species (Casal 2006; Sala, Chapin & Armesto 2000; Vörösmarty et al. 2010; Welcomme 1984), arose from these problems such as increased predation on native freshwater prey fish species and inter-specific competitions among herbivorous and omnivorous fish species, overcrowing and stunting, genetic degradation, proliferation of alien parasites and uncommon diseases, extinction of existing indigenous species and disturbance of freshwater ecosystems' stability (Amundsen et al. 2009; Bedarf et al. 2001; Gaygusuz, Tarkan & Gaygusuz 2007; Johnson, Bossenbroek & Kraft 2006). In Malaysia, recent field surveys and literature estimated a total of 42 alien fish species had been recorded as being introduced intentionally and unintentionally to Malaysian waters (Khairul Adha 2012; Khairul Adha, Yuzine & Aziz 2013). Increasing Malaysian population has led to the increment of invasive fish intentional farming, such as the Nile Tilapia, *Oreochromis niloticus*, as a cheap protein source since native fish resources alone were unable to meet the needs of local populations. Some of these farmed invasive fish species eventually escaped from caged cultures, established in an abandoned mining pool and reservoirs, and eventually established their populations in wild freshwater ecosystems (Khairul Adha 2012; Khairul Adha, Yuzine & Aziz 2013). Additionally, tilapias were the third most farmed fish around the world after carps and salmonids, accounting for at least 4% of global aquaculture production (FAO 2010). The presence of the popular aquarium fish, the Algae Suckermouth Catfishes, *Hypostomus plecostomus*, and the large piscivorous Peacock Bass, *Cichla ocellaris*, in rivers and ex-mining lakes, indicating that these two species had well established and self-maintained locally (Khairul Adha 2012; Khairul Adha, Yuzine & Aziz 2013).

Every effort should be taken to manage or eradicate the occurrence of these problematic invasive alien fish species from Malaysia's water bodies. Although prevention of importation of listed alien fish species, as well as increased firm law enforcements such as Fisheries Act 1985 and MAQIS Act 2011 had positive effects in managing invasive alien fish species and protecting native fish diversity (Khairul Adha, Yuzine & Aziz 2013), total eradication of invasive alien fish species without affecting native fish species after their establishment is usually not possible (Lodge et al. 1998). Physical eradication requires a long-term investment of human resources and chemical control has the risks to affect and eradicate native fish species along with the intended alien fish species. Several alien fish species have been resistant to chemicals, as well as has environmental risks if involved in highly toxic chemicals. At the same time, not much biological control can be commenced effectively without affecting native fish species since very little information is available for specific parasites and pathogens that can affect the populations of invasive alien fish species (Haubrock et al. 2018; Wittenberg & Cock 2001).

Another control method known as invasivorism, a consumer-based control method of invasive species, where mass-harvesting of highly invasive fish species done by local authorities and supply these wild-caught invasive fish species as an additional protein source to the community, only seemed to be effective to palatable fish species (Bouska et al. 2020) such as Nile Tilapia. However, more palatable and domesticated red strains of tilapia were increasingly farmed (Pullin et al. 1997), further reducing the probability of these wild Nile Tilapia being caught or collected for protein sources. In addition, invasivorism's applications were also ineffective against game fishes such as Peacock Bass and wild Algae Suckermouth Catfish, which can be considered unpalatable.

To date, there was no current revision on the management methodologies to mitigate the negative effects of the wild established invasive fish species, which have become the permanent part of the fish community in Malaysia's lentic and lotic water bodies. Management efforts of these long-established invasive fish species were considered exhaustive, time-consuming, not profitable, yet difficult and costly (Canonico et al. 2005; Iongh & Zon 1993). One of the avenues to incite the progress for managing the established invasive fish species was by converting these wild captured invasive fish species into practical, useful, yet profitable products, such as organic fertilizers. Fish-based fertilizers were considered one of the common and oldest forms of fertilizer elements in organic or green agriculture systems worldwide, rich in minerals, trace elements, nutrients and amino acids (Erkan, Selcuk & Ozden 2010). It is suitable for most agricultural applications (McNeill, Blanc & Rochers 2008; Nurul Ulfah, Mohd Farid & Adzemi 2015) and is proposed as supplementary amendments to the basic inorganic NPK compound fertilizers due to the instability of maritime or marine wastes (Susanto 2015). The implementations of fish-based organic fertilizers derived from widely spread and established invasive fish species could be more sustainable and beneficial to the environment compared to the traditional fish-based fertilizers derived from marine trash fishes (MTF) (Aranganathan & Radhika Rajasree 2016) that were converted into organic fish silages (Horn, Aspmo & Eijsink 2007). Heavy dependences on maritime or marine products resulted from increased overfishing activities to find economically valuable fishes, and collecting less valuable trash fishes for organic fertilizer sources could negatively affect the marine ecosystems' stabilities (Argüello 2020; Daskalov 2002; Daskalov et al. 2007). Similarly, heavy dependence on synthetic chemical fertilizers could be hazardous to the environment yet costly to most low-income farmers (Malaysian Agricultural Digest 2013). Hence, the applications of the established invasive fish species within lentic and lotic water bodies in Malaysia could be observed as a novel avenue for obtaining new sources of fish-based fertilizers without sole dependence on maritime or marine wastes. Therefore, this study was conducted to evaluate the efficacy of several established wild invasive alien fish species found in Malaysia (*C. ocellaris*, *O. niloticus*, and *H. plecostomus*) as potential new sources of fish-based organic fertilizers.

Materials and Methods

STUDY SITES

Wild Nile Tilapia (*O. niloticus*) and Algae Suckermouth Catfish (*H. plecostomus*) are the invasive fish samples caught and collected at Tasik Harapan, Universiti Sains Malaysia (USM), Pulau Pinang (5°21'14.99"N 100°18'3.00"E). Meanwhile, wild Peacock Bass (*C. ocellaris*) were collected at Tasik Raban, Lenggong, Perak (4°59'49.1"N 100°57'01.2"E). For all invasive fishes involved, 20 adult-sized fishes were chosen as representatives for each invasive fish species, and any captured small juveniles or fry were removed from the standard size fishing nets employed.

LABORATORY PROCESSES OF FISH SAMPLES

All of the captured and collected invasive fish species were brought to the laboratory of the School of Biological Sciences, G08 Building, USM, stored a few days in a chest freezer before thawed for further laboratory processing. The fishes' body parts (mostly muscles) were cut into pieces, where the internal organs, including the liver, heart, intestines and gills, were separated. The aforementioned internal organs were washed thoroughly and boiled with deionized water using the Sincero magnetic induction stable cooker at a fixed induction temperature of 60 °C for 50 minutes for slow boiling to kill any existing micro-organisms, and at the same time preserving any visceral or internal organs' fatty tissues. Fishes' muscles were not boiled together to avoid any contamination. The cut fishes' body parts were also boiled for the same purpose. Then, all body parts of the fish were oven-dried at 75 °C for six to seven hours until all the remaining water content in the fishes' muscles and internal organs were completely vaporized before being let cooled in a chiller for 4 hours.

The prepared fishes' muscles and internal organs were mixed together into a heavy-duty grinder, and they were homogenized and grounded until turning into coarse powders for exactly five to six minutes. The coarse fish powder for each invasive fish species were further made to be finer by employing a milling blender. The fine fish powders were then stored carefully in air-tight plastic containers, and silica gels packets were inserted. The prepared fine fish powders were brought to the School of Biological Sciences' Plant House, L14 Building, for further tests of the fine fish powders on the growth of selected vegetable species.

PROXIMATE ANALYSIS OF FERTILIZERS

For each invasive fish species, 250 g of fine fish powders, as well as 250 g NPK 15:15:15 compound fertilizer, were packed in durable vacuum sealer bags. The fine fish powder samples were submitted to Food Quality and Safety Research & Development (UNIPEQ UKM-MTDC Pvt. Ltd.), Universiti Kebangsaan Malaysia (UKM) for the determination of the percentage of trace Nitrogen (N %w/w), Phosphorus (P mg/kg) and Potassium (K mg/kg) as plant primary macronutrients, as well as trace Calcium (Ca mg/kg) and Magnesium (Mg mg/kg) as plant secondary macronutrients. Determination of trace Nitrogen (N %w/w) followed the standard Kjeldahl method, for minerals (Calcium, Magnesium, and Potassium, mg/kg) followed the Atomic Absorption Spectrophotometric (AAS) method and for Phosphorus (P mg/kg) followed the Microwave Plasma Atomic Emission Spectrophotometers (MP/AES) method (Latimer 2016).

EFFICACY TESTING OF THE FISH POWDER FERTILIZERS

Soils for the planting media were gathered from the School of Biological Sciences' Plant House (L14

Building) (5°21'26.4"N 100°17'39.9"E). Polybag of the size 10 cm × 12 cm was chosen in this study. The soils used in this study were first sterilized using heat treatment in the heating microwave oven at 105 °C for 20 minutes to kill any harmful microorganisms present in the soil samples. Then, the soils were sieved using a 1.0 mm sieve and were put into the chosen polybags, weighed accurately around 1 kg. One kilogram of the pre-prepared soil sample was carefully vacuum-packed and submitted to Allied Chemists Laboratory Pvt. Ltd. for detailed soil profiling analyses, including pH (6.4), Exchangeable Ca (34.5 mg/L, highest), Exchangeable K (15.5 mg/L, moderate), Exchangeable Mg (4.0 mg/L, lowest), Cation Exchange Capacity/CEC (5.3 meq/100 g), Total Organic Carbon (0.4 %w/w), and Total Organic Matter (8.9 %w/w). All following standard proximate analytical methodologies by Latimer (2016).

Two common vegetable species, the semi-aquatic water spinach, *Ipomoea aquatica* and the common terrestrial spinach, *Spinacia oleracea* were chosen in determining the efficacy of the fish powder fertilizers on their growth. The seedlings of both vegetables were first pre-germinated using germination containers, applied with deionized water and covered with black plastic bags to ensure successful germination. Fine cotton wool was chosen as the medium for the germination of both vegetable seedlings. After reaching around 5 cm of height, the seedlings were carefully moved into the prepared polybags. One polybag contained six seedlings for each vegetable species.

The polybags containing the vegetables' seedlings were watered with deionized water twice a day. Each watering involved 100 mL of deionized water. As a positive control for this study, the synthetic chemical NAFAS NPK compound fertilizer 15:15:15 was chosen and compared to the prepared fish powder fertilizers. There were five experimental replicates represented by the polybags for each fertilizer type as treatment, including a passive control with no fertilizer applied. All experimental treatments were arranged following the standard Completely Randomized Design (CRD). Five grams of each fertilizer type was applied to each polybag every week. The progress on the growth (heights, leaf width, and stalk width) of both vegetables were observed for seven weeks, where the growth of the vegetables was recorded weekly.

The heights of both vegetables were determined using a metal ruler and marked with white threads. A red permanent marker was used to permanently mark the basal section of the vegetables' stalk nearest to the soil's surface as a standard initial line of reference for each height measurement. Leaf width and stalk width measurements were determined by implementing a 300 mm digital caliper with 0.02 mm deviation. Readings for all measurements were recorded five times per seedling per polybag and per week. The average readings were used for statistical analyses to determine the efficacy of the fish powder fertilizers on the growth of the chosen vegetables.

STATISTICAL ANALYSES

Two-Sample Independent *t*-tests with Bootstrapping were run to determine differences among different levels of proximate chemical compositions for all fertilizer types. Two-Way ANOVA with Bootstrapping for the fixed effect/factor/source of variation involving fertilizer types and vegetable species on the growth parameters of the tested vegetables was run employing IBM SPSS Statistics Software version 26.0.0.0 64-bit edition. Kolmogorov-Smirnov Normality test was chosen for testing the normality of the dependent variables such as heights (mm), leaf width (mm) and stalk width (mm). Since the variances were not homogenous or homoscedastic, Welch Robust Test for the Equality of Means (Welch's Test or Welch's ANOVA) was chosen for the statistical analyses and with Games-Howell post hoc tests accommodation of unequal sample sizes and unequal sample variances. Hypotheses testing was set at a 95% of confidence level ($\alpha = 0.05$).

RESULTS

PROXIMATE ELEMENTAL CHEMICAL ANALYSES ON FERTILIZER TREATMENTS

For primary macronutrients, mean elemental Nitrogen (N) percentage (N %w/w), NPK 15:15:15 compound fertilizer recorded significantly the highest (15.81 ± 0.43, $p<0.05$) compared to other fish powder fertilizers. Among the fish powder fertilizers, *C. ocellaris* recorded the highest elemental N (10.57 ± 0.03, $p<0.05$), followed by *O. niloticus* (8.89 ± 0.02, $p<0.05$) and *H. plecostomus* (7.68 ± 0.27, $p<0.05$). For mean elemental Phosphorus (P) (P mg/kg), NPK 15:15:15 compound fertilizer recorded significantly the highest (38,928.65 ± 215.34, $p<0.05$) compared to all three fish powder fertilizers. Among these, *H. plecostomus* recorded the highest elemental P (30,562.09 ± 197.11, $p<0.05$), followed by *O. niloticus* (27,854.93 ± 37.15, $p<0.05$) and the lowest recorded by *C. ocellaris* (26,015.06 ± 116.82, $p<0.05$). Mean elemental Potassium (K) (K mg/kg) showed that NPK 15:15:15 compound fertilizer recorded significantly the highest (129,222.55 ± 845.48, $p<0.05$). Among the fish powder fertilizers, *C. ocellaris* recorded the highest elemental K (28,909.15 ± 32.56, $p<0.05$), followed by *O. niloticus* (20,694.09 ± 505.53, $p<0.05$) and the lowest recorded by *H. plecostomus* (14,696.13 ± 11.50, $p<0.05$) (Table 1).

For secondary macronutrients, mean elemental Calcium (Ca) (Ca mg/kg), NPK 15:15:15 compound fertilizer significantly recorded the highest (2,430.76 ± 25.19, $p<0.05$), and among the fish powder fertilizers, *H. plecostomus* recorded the highest elemental Ca (6,984.48 ± 26.20, $p<0.05$), followed by *O. niloticus* (6,766.18 ± 23.82, $p<0.05$), with *C. ocellaris* recorded the lowest (5052.92 ± 57.97, $p<0.05$). Mean of elemental Magnesium (Mg) (Mg mg/kg) in NPK 15:15:15 compound fertilizer recorded significantly the highest (1657.35 ± 7.37, $p<0.05$), and among the fish powder fertilizers, *C. ocellaris* recorded the highest elemental Mg (1496.66 ± 3.99, $p<0.05$), followed by *O. niloticus* (1,378.42 ± 11.71, $p<0.05$) and *H. plecostomus* recorded the lowest (1,294.60 ± 3.86, $p<0.05$) (Table 2).

TABLE 1. Mean ± S.E. of trace element of primary plant macronutrients, Nitrogen (N %w/w), Phosphorus (P mg/kg), and Potassium (K mg/kg) for all fish powder fertilizers and the NPK 15:15:15 compound fertilizer. Different alphabetic elements showed significance differences of trace elements ($\alpha = 0.05$)

	N (% w/w)	P (mg/kg)	K (mg/kg)
NPK 15:15:15	$15.81 \pm 0.43_a$	$38928.65 \pm 215.34_a$	$129222.55 \pm 845.48_a$
C. ocellaris	$10.57 \pm 0.03_b$	$26015.06 \pm 116.82_b$	$28909.15 \pm 32.56_b$
O. niloticus	$8.89 \pm 0.02_c$	$27854.93 \pm 37.15_c$	$20694.09 \pm 505.53_c$
H. plecostomus	$7.68 \pm 0.27_d$	$30562.09 \pm 197.11_d$	$14696.13 \pm 11.50_d$

TABLE 2. Mean ± S.E. of trace element of secondary plant macronutrients, Calcium (Ca mg/kg), and Magnesium (Mg mg/kg) for all fish powder fertilizers and the NPK 15:15:15 compound fertilizer. Different alphabetic elements showed significance differences of trace elements ($\alpha = 0.05$)

	Ca (mg/kg)	Mg (mg/kg)
NPK 15:15:15	$2430.76 \pm 25.19_a$	$1657.35 \pm 7.37_a$
C. ocellaris	$5052.92 \pm 57.97_b$	$1496.66 \pm 3.99_b$
O. niloticus	$6766.18 \pm 23.82_c$	$1378.42 \pm 11.71_c$
H. plecostomus	$6984.48 \pm 26.20_d$	$1294.60 \pm 3.86_d$

EFFICACY OF APPLIED FERTILIZERS ON GROWTH PARAMETERS OF SELECTED VEGETABLES

Two-Way ANOVA for the heights (mm) based on the factors of different fertilizers and vegetable species showed significant differences among fertilizer types and vegetable species ($p<0.05$), but there was no interaction between the two factors ($p>0.05$). *I. aquatica* showed that application of *C. ocellaris* powder recorded the highest heights, but not significantly different from other fertilizers (263.74 ± 12.29 mm, $p>0.05$), followed by the

NPK 15:15:15 fertilizer (236.64 ± 10.60 mm, $p>0.05$), *H. plecostomus* (222.49 ± 9.51 mm, $p>0.05$) and *O. niloticus* (213.15 ± 10.06 mm, $p>0.05$). All fertilizers significantly different from the control treatment (135.57 ± 2.18 mm, $p<0.05$). *S. oleracea* showed non-significant differences for all applied fertilizers, where *C. ocellaris* recorded the highest heights (166.35 ± 9.46 mm, $p>0.05$), followed by the NPK 15:15:15 fertilizer (164.30 ± 9.85 mm, $p>0.05$), *H. plecostomus* (161.82 ± 11.07 mm, $p>0.05$) and *O. niloticus* (156.51 ± 9.24 mm, $p>0.05$). All fertilizers significantly different from the control treatment (82.51 ± 2.47mm, $p<0.05$) (Figure 1).

Leaf widths (mm) based on the factors of different fertilizers and vegetable species showed significant differences among different fertilizer types and vegetable species ($p<0.05$), but there was no interaction between the two factors ($p>0.05$). *I. aquatica* showed that application of *C. ocellaris* recorded the widest leaf widths (14.82 ± 0.66 mm, $p>0.05$) but was not significantly different from *O. niloticus* (13.03 ± 0.65 mm, $p>0.05$) and followed by the NPK 15:15:15 (12.67 ± 0.59 mm, $p>0.05$). All three fertilizers mentioned above were significantly different from *H. plecostomus* (10.74 ± 0.57 mm, $p<0.05$) and the control treatment (9.29 ± 0.36 mm, $p<0.05$). *S. oleracea* showed that *C. ocellaris* recorded the widest leaf widths (21.08 ± 1.53 mm, $p> 0.05$) but was not significantly different from the other fertilizers, followed by the NPK 15:15:15 (20.68 ± 1.41 mm, $p>0.05$), *H. plecostomus* (19.50 ± 1.24 mm, p>0.05) and *O. niloticus* (18.88 ± 1.17 mm, $p>0.05$). All fertilizers were significantly different from the control treatment (10.53 ± 0.40 mm, $p<0.05$) (Figure 2).

Stalk widths (mm) based on different fertilizers and vegetable species showed significant differences among different fertilizer types and vegetable species ($p<0.05$), but there was no interaction between the two factors ($p>0.05$). *I. aquatica* showed that application of *C. ocellaris* recorded the widest stalk widths (3.06 ± 0.10 mm, $p>0.05$) and was not significantly different from the other two fish powder fertilizers of *H. plecostomus* (2.92 ± 0.07 mm, $p>0.05$) and *O. niloticus* (2.82 ± 0.07 mm, $p>0.05$). All three fish powder fertilizers were significantly different from the NPK 15:15:15 fertilizer (2.64 ± 0.06 mm, $p<0.05$) and the control treatment (2.58 ± 0.04 mm, $p<0.05$). *S. oleracea* showed that application of *C. ocellaris* recorded the widest stalk widths (2.89 ± 0.17 mm, $p>0.05$) and was not significantly different from other applied fertilizers. This followed by *H. plecostomus* (2.77 ± 0.14 mm, $p>0.05$), *O. niloticus* (2.70

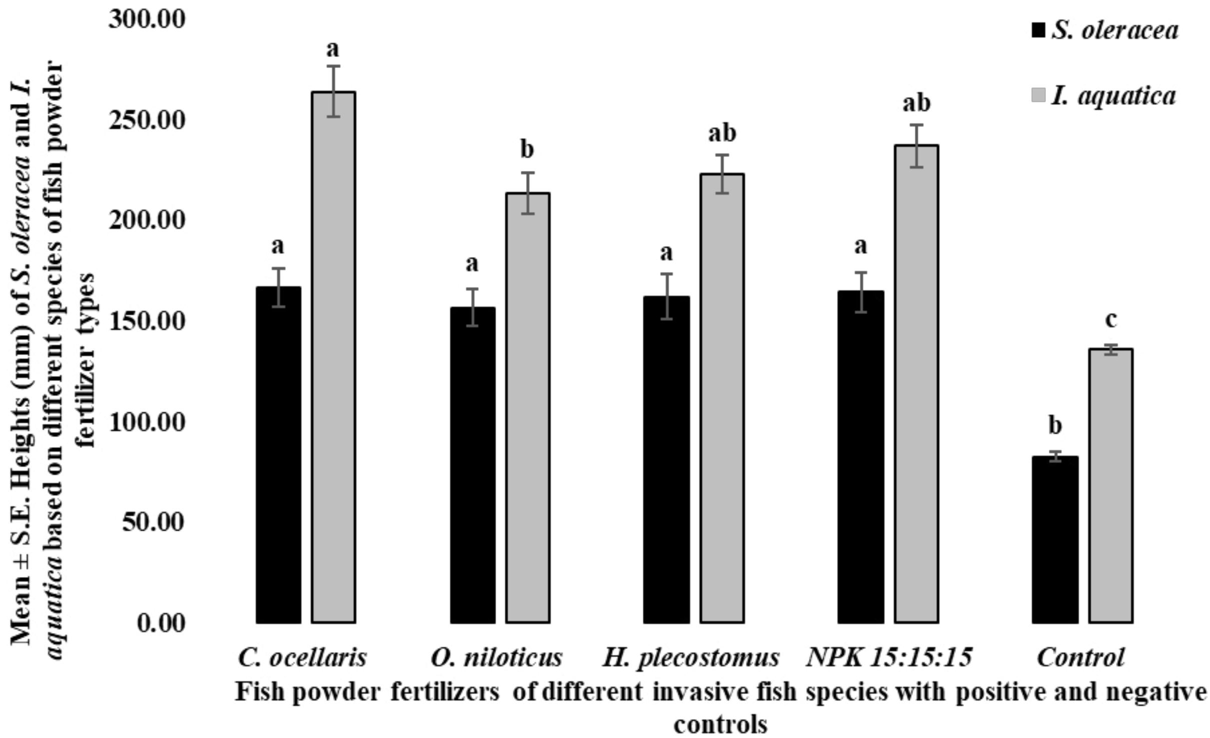

FIGURE 1. Mean ± (S.E.) heights of spinach (*Spinacia oleracea*) and water spinach (*Ipomoea aquatica*) based on different fertilizer types applied. Different alphabetic elements showed significant heights' difference based on Games-Howell post hoc tests (α = 0.05)

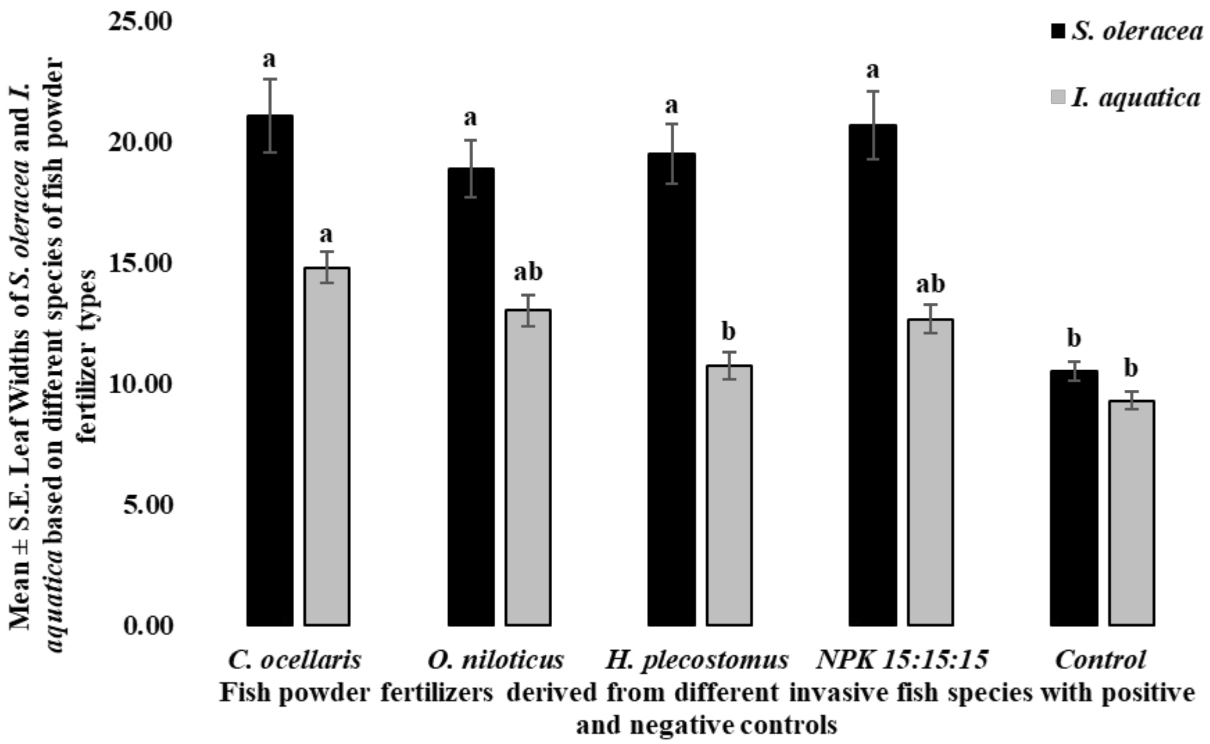

FIGURE 2. Mean ± (S.E.) leaf widths of spinach (*Spinacia oleracea*) and water spinach (*Ipomoea aquatica*) based on different fertilizer types applied. Different alphabetic elements showed significant leaf widths' difference based on Games-Howell post hoc tests ($\alpha = 0.05$)

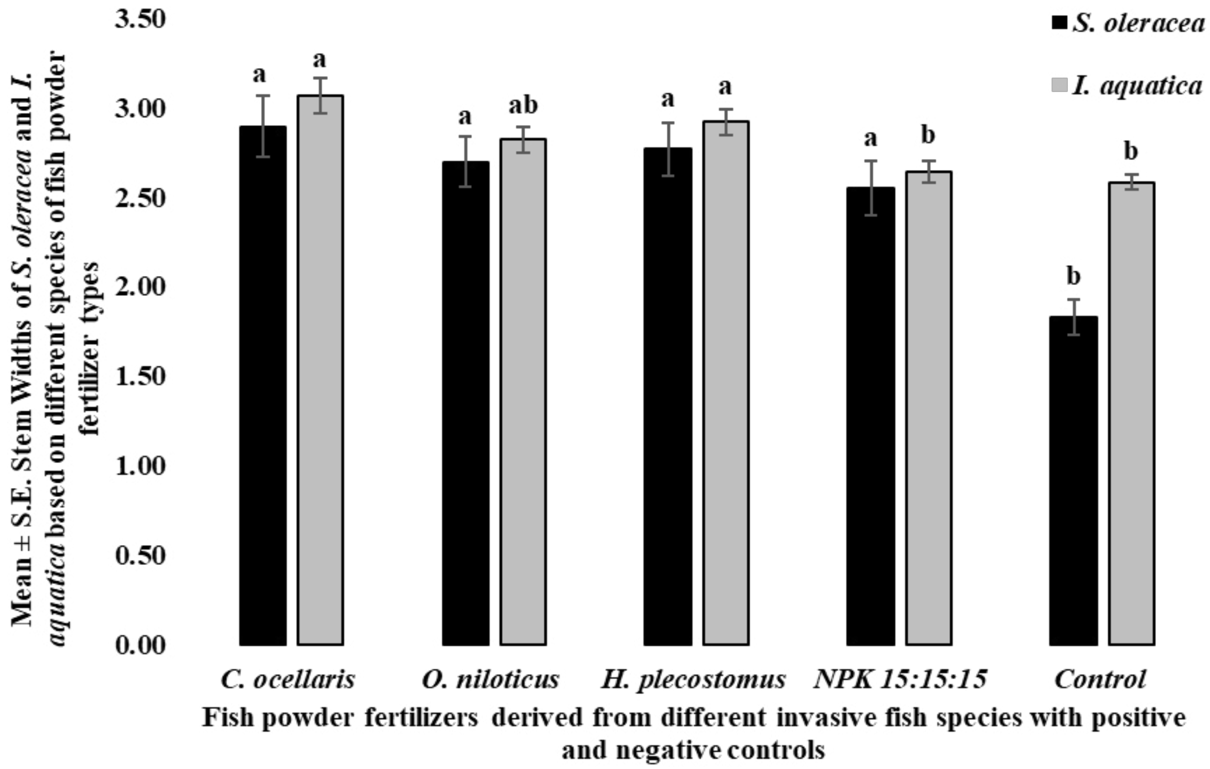

FIGURE 3. Mean ± (S.E.) stalk widths of spinach (*Spinacia oleracea*) and water spinach (*Ipomoea aquatica*) based on different fertilizer types applied. Different alphabetic elements showed significant stalk widths' difference based on Games-Howell post hoc tests ($\alpha = 0.05$)

± 0.14 mm, $p>0.05$) and the lowest by the NPK 15:15:15 fertilizer (2.55 ± 0.15 mm, $p>0.05$). All of the applied fertilizers were significantly different from the control treatment (1.83 ± 0.10 mm, $p<0.05$) (Figure 3).

DISCUSSION

Based on our results, Peacock Bass, *C. ocellaris*, is the only fish powder fertilizer that showed obvious effects on all measured growth parameters of both semi-aquatic *I. aquatica* and terrestrial *S. oleracea* vegetables. Additionally, it was not significantly different from the NPK 15:15:15 compound fertilizer. The NPK 15:15:15 compound fertilizer recorded the highest average of total Nitrogen (N %w/w), Phosphorus (P mg/kg), and Potassium (K mg/kg). It was initially hypothesized that this fertilizer would produce highest growth of tested vegetables compared to other fish powder fertilizers. Heights (mm) and leaf widths (mm) were among the two most affected measured growth parameters, especially for the semi-aquatic vegetable, *I. aquatica*, compared to the terrestrial vegetable, *S. oleracea*. All three fertilizers, including the NPK 15:15:15 and both fish powder fertilizers derived from *O. niloticus* and *H. plecostomus*, showed somewhat reduced heights (mm) and leaf widths (mm), compared to the fish powder fertilizer derived by *C. ocellaris*. This possibly could be explained via the synergisms or antagonisms of cation uptake by the root systems, including the excess or deficiency of cations existing in the soil used in this study, interacted with the elements available in the applied fertilizers that affected water balance and water-use efficiency of *I. aquatica* vegetable.

Water spinach vegetable, *I. aquatica* is a semi-aquatic vegetable that requires high moisture soil for optimal growth (Tiwari & Chandra 1985). The soil used in this study was Magnesium (Mg)-deficient, moderately high in Potassium (K) and high in Calcium (Ca). This soil coincided with the findings by Kong (1994), where the order of abundance of cations in the pore fluids was $Na^+ >> Ca^{2+} > K^+ > Mg^{2+}$, and of to be very high dispersion potential for the existing cations. For the NPK 15:15:15 fertilizer, when sandy-type (Chigira et al. 2011) and low CEC soil were applied with high ammonium (NH_4^+) or K^+ fertilizers, these two cations intensely competed for the absorption of Mg^{2+} ions. Ammonium ions (NH_4^+) have been shown to decrease Mg uptake through its acidifying characteristic when assimilated and ionic competitions at the adsorbing surfaces of the roots (Mulder 1956). This further explains that although NPK 15:15:15 fertilizer recorded the highest Mg concentration of all tested fertilizers in this study, the available Mg ions (Mg^{2+}) were not readily absorbed and possibly leached and depleted down within the soil rhizosphere. These two cations could further lead to Mg^{2+} ions depletion and leaching out of the low CEC soil, exhibiting nutrient antagonism (Guo et al. 2016; Mulder 1956). This condition will not usually occur if the exchangeable Mg is higher than the exchangeable K, where plants developed a specialized K-transport system in the root cells to ensure sufficient K uptake. For Mg, the transporters are non-specific and it can be passed by other cations such as K. Therefore, when the concentration of K in the soil-root interface is high, together with the application of the NPK 15:15:15 fertilizer that contains the highest concentration of K, the sufficient Mg uptake by *I. aquatica* would be limited. This suggests that excessive K concentration in the roots might depress the rate of net Mg translocation from roots to shoots (Senbayram et al. 2015).

In most studies, a high rate of K fertilizers will increase the stomatal conductance, which will induce water loss due to prolonged stomatal openings for the high diurnal transpiration rate and low water absorption, and increase production of organic acids that accumulated in the root exudates. This further depletes stem water content for the removal of excess organic acids-based root exudates into the rhizospheres (Benlloch-González et al. 2010; Chen et al. 2013). Most root exudates in the form of organic acids inhibit the binding of soil structures. In contrast, root exudates comprised of sugars in the form of polysaccharides produced by plants with normal stomatal conductance could gel the soil together and create a more stable soil structure around roots and seeds (Naveed et al. 2017). Hence, reducing the ability of the soil to retain water and viable cation such as Mg. This prolonged condition causes inefficient water usage for *I. aquatica* since this vegetable requires high moisture content. Most of the high organic acids root exudates and stem water content are possibly leached down off the low CEC sandy soil. These soil types usually made the leached root exudates nutrients and the stem water content irretrievable. Reversible root exudates' nutrients require optimal water levels in the rhizospheres since it serves as the medium for reabsorption processes (Gargallo-Garriga et al. 2018; Preece et al. 2018). High K with high probability of low Mg absorption, as depicted by the NPK 15:15:15 fertilizer also posed the high risks of unproductive water loss at night caused by increased nocturnal respiration, where most Mg deficient plants depicted night-time water loss of more than 35% of daytime or diurnal transpiration (Tränkner et al. 2016). Together, these three conditions: increased diurnal transpiration, root exudations with high organic acids and night-time respiration are accounted for the inefficient water-use for *I. aquatica*. Furthermore, the low CEC soil that leached most of the soil water and the stem water content from the excessive root exudates led to dry soils and less water in the stem structure, contributing to physiological imbalances and affected overall growth (Guo et al. 2016; Ridolfi et al. 1996; Senbayram et al. 2015).

Similarly, comparison of *C. ocellaris* fish powder fertilizer with the other two fish powder fertilizers derived from *O. niloticus* and *H. plecostomus* suggested that the latter two fertilizers recorded significantly higher Ca concentrations. Moreover, the soil used in this study also recorded the highest exchangeable cation involving Ca, producing the highest possibility of Ca toxicity in terms of imbalances of water-use efficiency for *I. aquatica*. Unlike K, Ca transporters are non-specific, similar to Mg, but Ca bound more competitively and antagonistically against Mg to the root transport systems and have the higher affinity to be absorbed compared to Mg according to concentration gradient (Sembayram et al. 2015), producing similar effects of Mg deficiency. In many studies, Mg uptake has been repeatedly shown to be lower in soils with high concentrations of Ca in the soil solution (Guo et al. 2016). Excessive Ca produced several adverse effects on plant internal physiological balances, including reduced stomatal conductance (Ruiz et al. 1993; Tränkner et al. 2016). This is similar to Mg deficiency, which reduced the assimilation of diurnal CO_2 gaseous exchange. Although it seemed advantageous to plant water-use efficiency, the decreased water vapor pressure resulting from reduced stomatal openings resulted in the increased internal stem water levels, and pressuring for removal of excess water via the route of root exudations. Root exudations with the effects of excessive Ca into the rhizosphere system further led to the loss of soil water and exuded water from the root exudation process. Many studies proved that excessive Ca in the rhizosphere could pull more water out of the roots into the soil. The condition could be even more severe when involving low CEC soil where this water loss was irretrievable, as the loss of water from the root might exceed the conservation of water via stomatal closure (Park, Moon & Waterland 2020). Plants with reduced CO_2 gaseous exchange had pronounced problems related to phloem loading of polysaccharides, thus, reducing the absorption of more CO_2 gases. Hence, the root exudates expelled were mostly water with low polysaccharides and organic acids, and the gelling effect to bind soil structures together might not successfully happen (Naveed et al. 2017). Under water stress conditions, some plants exudated higher ratios of organic acids than polysaccharides (Preece et al. 2018), contributing to the reduced overall biomass growth of the plants (Tränkner et al. 2016). Since *I. aquatica* is a semi-aquatic plant, water in the form of moisture was as important as the presence of trace nutrients.

It was postulated that the fish powder fertilizer derived from *C. ocellaris* has more advantages compared to other fertilizers. The *C. ocellaris*-based fertilizer consists of moderate K concentration compared to the NPK 15:15:15 fertilizer, while at the same time, it consists of lower Ca and moderately higher Mg compared to fish powder fertilizers derived from *O. niloticus* and *H. plecostomus.* The fish powder fertilizer derived from *C. ocellaris* already has a higher concentration of Mg to be less antagonized for the uptake and absorptions processes by the moderate concentrations of both K and Ca ions. In other words, there was a higher probability that the Mg applied in *C. ocellaris* fish powder fertilizer to be absorbed by both *I. aquatica* and *S. oleracea* vegetables. Absorption of Mg^{2+} by both *I. aquatica* and *S. oleracea* produced positive results in terms of growths. Opposite to the Mg deficiency symptoms in the NPK 15:15:15-, *O. niloticus*- and *H. plecostomus*-treated vegetables, there were lower risks of either excessive or reduced stomatal conductance. Hence, the net carbon gains and water loss via the evapotranspiration process at the surface of the leaves were balanced (Tränkner et al. 2016). At the same time, the risk of night-time respiration and over excessive root exudations that could produce unproductive water loss is lowered, more importantly for *I. aquatica*.

Many studies have proved that higher Mg concentration in plant tissues promotes better growth. Mg and P showed positive synergism as both elements were heavily involved in various photosynthetic processes. Meanwhile, low Mg concentration has been reported to reduce N conversion efficiency. Increasing Mg concentration would increase N conversion efficiency, indicating that the Mg:P synergistic mechanism supports photosynthetic N use and ultimately improves growth (Weih et al. 2021). Yousaf et al. (2021) also showed that the combined higher concentration of both N- and Mg-based fertilizers significantly produce the highest heights, the number of leaves, leaf widths, leaf weights, chlorophyll contents and total biomass compared to separate N fertilizer or Mg fertilizer. This proves that Mg was an elemental synergist with N in producing improved growths. The fact that the N source in *C. ocellaris* fish powder fertilizer was not in the form of inorganic N such as NH_4^+ ions, rather organic forms such as amino acids could further improve growths without compromising the reduced uptake of Mg^{2+} ions (Senbayram et al. 2015).

Conclusion

For the purpose of managing the already established and widespread invasive fish species in Malaysia's lentic and lotic water bodies, it is recommended to focus on the carnivorous or piscivorous invasive fish species such as *C. ocellaris* since these fishes, as proved in this study, have much better tolerable concentrations of K and Ca, more importantly for semi-aquatic plants such as *I. aquatica* that relied on effective water-use efficiency, as well as with higher Mg concentration that worked together with N and P as a positive synergist, producing better growths, compared to the inorganic NPK compound fertilizer with too high K concentration, and the other two fish powder fertilizers derived from *O. niloticu*s and *H. plecostomus* with higher Ca concentration, and all these three fertilizers inhibited the uptake, absorptions, and fully utilizations of Mg ions (Mg^{2+}) supplied in respective fertilizers. The use of the predatory or piscivorous invasive fish species can reduce the overdependence of transformed trash fish wastes from maritime or marine industries as currently the only source of fish-based organic fertilizer.

ACKNOWLEDGEMENTS

We would like to address our highest gratitude to the School of Biological Sciences, Universiti Sains Malaysia (USM), for providing us Laboratory 333B and Laboratory 321 (G08 Building), and all the related laboratory equipments for the organic and inorganic fertilizer processing, as well as the Plant House (L14 Building), for vegetables' growth experimentations. We would like to distinguish UNIPEQ UKM-MTDC Pvt. Ltd. and Allied Chemists Laboratory Pvt. Ltd. for providing us fast, feasible, and excellent raw organic, inorganic fertilizers', and soil proximate laboratory analyses.

REFERENCES

Amundsen, P.A., Siwertsson, A., Primicerio, R. & Bohn, T. 2009. Long-term responses of zooplankton to invasion by a planktivorous fish in a subarctic watercourse. *Freshwater Biology* 54: 24-34.

Aranganathan, L. & Radhika Rajasree, S.R. 2016. Bioconversion of marine trash fish (MTF) to organic liquid fertilizer for effective solid waste management and its efficacy on tomato growth. *Management of Environmental Quality: An International Journal* 27(1): 93-103.

Argüello, G. 2020. Environmentally sound management of ship wastes: Challenges and opportunities for European ports. *Journal of Shipping and Trade* 5: 12.

Bedarf, A.T., McKaye, K.R., Van Den Berghe, E.P., Perez, L.J.L. & Secor, D.H. 2001. Initial six-year expansion of an introduced piscivorous fish in a tropical Central American lake. *Biological Invasions* 3: 391-404.

Benlloch-González, M., Romera, J., Cristescu, S., Harren, F., Fournier, J.M. & Benlloch, M. 2010. K^+ starvation inhibits water-stress-induced stomatal closure via ethylene synthesis in sunflower plants. *Journal of Experimental Botany* 61(4): 1139-1145. doi:10.1093/jxb/erp379

Bouska, W.W., Glover, D.C., Trushenski, J.T., Secchi, S., Garvey, J.E., MacNamara, R., Coulter, D.P., Coulter, A.A., Irons, K. & Wieland, A. 2020. Geographic-scale harvest program to promote invasivorism of big-headed carps. *MDPI Fish Journal* 5(29): doi.10.3390/fishes5030029

Canonico, G.C., Arthington, A., McCrary, J.K. & Thieme, M.L. 2005. The effects of introducted tilapia on native biodiversity. *Aquatic Conservation: Marine and Freshwater Ecosystems* 15: 463-483.

Casal, C.M.V. 2006. Global documentations of fish introduction: the growing crisis and recommendations for action. *Biological Invasions* 8: 3-11.

Chen, Y., Yu, M., Zhu, Z., Zhang, L. & Guo, Q. 2013. Optimisation of potassium chloride nutrition for proper growth, physiological development, and bioactive component production in *Prunella vulgaris*, L. *PLoS ONE* 8(7): e66259. doi.10.1371/journal.pone.0066259

Chigira, M., Zainab, M., Sian, L.C. & Ibrahim, K. 2011. Landslides in weathered granitic rocks in Japan and Malaysia. *Bulletin of the Geological Society of Malaysia* 57: 1-6.

Daskalov, G.M. 2002. Overfishing drives a trophic cascade in the Black Sea. *Marine Ecology Progress Series* 225: 53-63.

Daskalov, G.M., Grishin, A.N., Rodionov, S. & Mihneva, V. 2007. Trophic cascades triggered by overfishing reveal possible mechanisms of ecosystem regime shift. *Proceedings of the National Academy of Sciences of the United States of America* 104(25): 10518-10523.

Erkan, N., Selcuk, A. & Ozden, O. 2010. Amino acid and vitamin composition of raw and cooked horse mackerel. *Food Analytical Methods* 3: 269-275.

Food and Agriculture Organization of the United Nations (FAO). 2010. *Introduced Species Facts Sheets.* Fisheries and Aquaculture Department. http://www.fao.org/fishery/introsp/9144/en. Accessed on 7 March 2006.

Gargallo-Garriga, A., Preece, C., Sardans, J., Oravec, M., Urban, O. & Peñuelas, J. 2018. Root exudate metabolomes change under drought and show limited capacity for recovery. *Nature - Scientific Reports* 8: 12696. doi.10.1038/s41598-018-30150-0

Gaygusuz, O., Tarkan, A.S. & Gaygusuz, C.G. 2007. Changes in the fish community of the Ömerli Reservior (Turkey) following the introduction of non-native gibel carp *Carassius gibelio* (Bloch 1782) and other human impacts. *Aquatic Invasions* 2: 117-120.

Guo, W., Nazim, H., Liang, Z. & Yang, D. 2016. Magnesium deficiency in plants: An urgent problem. *The Crop Journal* 4: 83-91. doi.org/10.1016/j.cj.2015.11.003

Haubrock, P.J., Criado, A., Monteoliva, A.P., Monteoliva, J.A., Santiago, T., Inghilesi, A.F. & Tricarico, E. 2018. Control and eradication efforts of aquatic alien fish species in Lake Caicedo Yuso-Arreo. *Management of Biological Invasions* 9(3): 267-278.

Horn, S.J., Aspmo, S.I. & Eijsink, V.G. 2007. Evaluation of different cod viscera fractions and their seasonal variation used in a growth medium for lactic acid bacteria. *Enzyme and Microbial Technology* 40: 1328-1334.

Iongh, H.H.D. & Zon, J.C.J.V. 1993. Assessment of impact of the introduction of exotic fish species in north-east Thailand. *Aquaculture and Fisheries Management* 24: 279-289.

Johnson, L.E., Bossenbroek, J.M. & Kraft, C.E. 2006. Patterns and pathways in the post-establishment spread of non-indigenous aquatic species: The slowing invasion of North American inland lakes by the zebra mussel. *Biological Invasions* 8: 475-489.

Khairul Adha, A.R. 2012. Diversity, ecology, and distribution of non-indigenous freshwater fish in Malaysia. Ph.D. Thesis, Universiti Putra Malaysia (Unpublished).

Khairul Adha, A.R., Yuzine, E. & Aziz, A. 2013. The influence of alien fish species on native fish community structure in Malaysian waters. *Kuroshio Science* 7(1): 81-93.

Kong, T.B. 1994. Engineering properties of granitic soils and rocks of Penang Island, Malaysia. *Bulletin of the Geological Society of Malaysia* 35: 69-77.

Latimer, G.W. 2016. *Official Methods of Analysis of AOAC International*. 20th ed. Gaithersburg, MD, USA: AOAC International. p. 3172.

Lodge, D.M., Stein, R.A., Brown, K.M., Covich, A.P., Bronmark, C. & Garvey, J.E. 1998. Predicting impact of freshwater exotic species on native biodiversity: Challenges in spatial scaling. *Australian Journal of Ecology* 23: 53-67.

Malaysian Agricultural Digest. 2013. *Chapter 16: Agricultural Chemicals*. pp. 155-163.

McNeill, A., Blanc, M. & Rochers, K.D. 2008. From sea to soil: Adding value to fish waste. *SPC Fisheries Newsletter* 126: 31-36.

Mulder, E.G. 1956. Nitrogen-magnesium relationships in crop plants. *Plant and Soil* 7: 341-376.

Naveed, M., Brown, L.K., Raffan, A.C., George, T.S., Bengough, A.G., Roose, T., Sinclair, I., Koebernick, N., Cooper, L., Hackett, C.A. & Hallett, P.D. 2017. Plant exudates may stabilize or weaken soil depending on species, origin, and time. *European Journal of Soil Sciences* 68(6): 806-816. doi.10.1111/ejss.12487.

Nurul Ulfah, K., Mohd Farid, M.A.L. & Adzemi, M.A. 2015. The effectiveness of fish selage as organic fertilizer on post-harvest quality of Pak Choy (*Brassica rapa* L. subsp. *chinensis*). *European International Journal of Science and Technology* 4(5): 163-174.

Park, S., Moon, Y. & Waterland, N.L. 2020. Treatment with calcium chloride enhances water deficit stress tolerance in Viola (*Viola cornuta*). *Horticulture Science* 55(6): 882-887.

Preece, C., Farré-Armengol, G., Llusià, J. & Peñuelas, J. 2018. Thirsty tree roots exude more carbon. *Tree Physiology* 38: 690-695. doi:10.1093/treephys/tpx163

Pullin, R.S., Palmares, M.L., Casal, C.V., Dey, M.M. & Pauly, D. 1997. Environmental effects of tilapias. In *Proceeding of the Fourth International Symposium on Tilapia in Aquaculture*, edited by Fitzsimmons, K. Northeast Regional Agricultural Engineering Service, USA. pp. 554-572.

Ridolfi, M., Roupsard, O., Garrec, J.P. & Dreyer, E. 1996. Effects of a calcium deficiency on stomatal conductance and photosynthetic activity of *Quercus robur* seedlings grown on nutrient solution. *Annals of Forest Science* 53(2-3): 325-335.

Ruiz, L.P., Atkinson, C.J. & Mansfield, T.A. 1993. Calcium in the xylem and its influence on the behaviour of stomata. *Philosophical Transactions of the Royal Society B* 341: 67-74.

Sala, O.E., Chapin, F.S. & Armesto, J.J. 2000. Global biodiversity scenarios for the year 2100. *Science* 287: 1770-1774.

Senbayram, M., Gransee, A., Wahle, V. & Thiel, H. 2015. Role of magnesium fertiliser in agriculture: Plant-soil continuum. *Crop and Pasture Science* 66: 1219-1229. doi.org/10.1071/CP15104

Susanto, I.R. 2015. Sustainable organic farming for environmental health: A social development model. *International Journal of Scientific and Technology Research* 4(5): 196-211.

Tiwari, N. & Chandra, V. 1985. Water spinach - its varieties and cultivation. *Indian Horticulture* 30(2): 23-24.

Tränkner, M., Jákli, B., Tavakol, E., Geilfus, C-M., Cakmak, I., Dittert, K. & Senbayram, M. 2016. Magnesium deficiency decreases biomass water-use efficiency and increases leaf water-use efficiency and oxidative stress in barley plants. *Plant Soil* 406: 409-423. doi.10.1007/s11104-016-2886-1

Vörösmarty, C.J., McIntyre, P.B., Gessner, M.O., Dudgeon, D., Prusevich, A., Green, P., Glidden, S., Bunn, S.E., Sullivan, C.A., Liermann, C.R. & Davies, P.M. 2010. Global threats to human water security and river biodiversity. *Nature* 467(7315): 555-561.

Weih, M., Liu, H., Colombi, T., Keller, T., Jäck, O., Vallenback, P. & Westerbergh, A. 2021. *Nature - Scientific Reports* 11: 9012. doi.org/10.1038/s41598-021-88588-8

Welcomme, R.L. 1984. International transfers of inland fish species. In *Distribution, Biology, and Management of Exotic Fishes*, edited by Courtenay Jr., W.R. & Stauffer Jr., J.R. Baltimore: Johns Hopkins University Press. pp. 22-40.

Wittenberg, R. & Cock, M.J.W. 2001. *IAS: A Toolkit of Best Prevention and Management Practices*. Wallingford: CAB International. p. 228.

Yousaf, M., Bashir, S., Raza, H., Shah, A.N., Iqbal, J., Arif, M., Bukhari, M.A., Muhammad, S., Hashim, S., Alkahtani, J., Alwahibi, M.S. & Hu, C. 2021. Role of nitrogen and magnesium for growth, yield, and nutritional quality of radish. *Saudi Journal of Biological Sciences* 28: 3021-3030. doi.org/10.1016/j.sjbs.2021.02.043

*Corresponding author; email: abukhary@usm.my