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Original Article

Effects of malachite green on growth and tissue accumulation in pak choy (*Brassica chinensis* Tsen & Lee)

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ABSTRACT

Reuse for agricultural purposes of aquaculture wastewater containing high levels of nutrients can be integrated into a water management strategy, in order to conserve water and alleviate water pollution problems. However, rather than nutrients, some contaminants in aquaculture wastewater may pose detrimental effects on plants being nourished. This study assessed the growth and accumulation of toxic substances of *Brassica chinensis* in response to Malachite Green (MG)-contaminated water. Plant seedlings were hydroponically grown with MG at 1 mg/L, 2 mg/L or 4 mg/L under ambient air conditions in the laboratory for 4 wk. Growth parameters—the number of leaves, plant height, leaf length and width, root length and dry mass of the plants—were compared with plants grown without MG (control). The concentrations at 2 mg/L and 4 mg/L affected the growth of the plants as measured by leaf length, plant height and leaf width generally to a lesser degree than the control plants and those grown at 1 mg/L MG ($p < 0.05$). The roots of plants were clearly affected by MG (average root length = 14.00 ± 1.17 cm, 14.50 ± 3.91 cm, 7.17 ± 1.52 cm and 6.58 ± 0.94 cm for plants from the control and treatments with MG at 1 mg/L, 2 mg/L and 4 mg/L, respectively, $p < 0.001$). The dry mass of treated plants (average dry mass = 1.22 ± 0.48 g/plant, 1.17 ± 0.27 g/plant and 0.86 ± 0.17 g/plant for treatments of MG at 1 mg/L, 2 mg/L and 4 mg/L, respectively) were lower than that of control plants (1.80 ± 0.73 g/plant) ($p < 0.001$). The increase in the oxalate content in the plant shoots suggested that the plants may accumulate substances that could be harmful to human health. Based on these results, it is proposed that the integration of hydroponic plant production with MG-contaminated water at a concentration not exceeding 1 mg/L can be applied without any reduction in the productivity of *B. chinensis*; however, the accumulation of toxic substances in plant tissues still needs to be identified.

Introduction

Malachite Green (MG), a triphenylmethane dye, is a multiple-use compound that is mainly used in textile industries and partly used in aquaculture in fungicides and ectoparasiticides (Srivastava et al., 2004; Fu et al., 2013). While the effects of MG on aquatic invertebrates and algae have been scarcely elucidated (Sudova et al., 2007), Hidayah et al. (2013) reported that MG in wastewater from either industry or aquaculture has been widely reported to be toxic to many kinds of fish with lethal effects at a concentration of less than 1 mg/L, with the dye and its derivatives being accumulated in aquaculture products such as fish, prawn and crab. It also possesses carcinogenic and genotoxic properties which pose a potential risk to humans and therefore, this dye has been banned in Europe, the USA and several countries (Srivastava et al., 2004). However, MG is still being used in some parts of the world because it is highly effective and easily available at low cost (Srivastava et al., 2004). It is also used domestically as a treatment for diseases of tropical fish and can be readily obtained by the public (Culp and Beland, 1996); hence, concern about its illegal use exists (Mitrowska et al., 2005). In Asian countries such as Bangladesh, MG has been reported to be used for the eradication of external parasites and fungal diseases in fish farming (Shamsuzzaman and Biswas, 2012). However, removal of MG from aquaculture wastewater has received little or no attention compared to other pollutants. Consequently, contamination of MG in aquaculture waste could be expected with harmful consequences to the surrounding environment.

Effluents from aquaculture usually contain high amounts of nutrients such as nitrogen (ammonia, nitrite and nitrate),

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phosphorus and organic compounds that either potentially cause algal bloom in receiving water (Miller and Semmens, 2002) or, if high enough, can support vegetable production (Yeo et al., 2004). To reduce water pollution problems, fishery industries in many countries including Thailand have been forced to treat their effluent in proper ways such as by the rational use of water and by the recovery of substances from wastewater (González, 1995). Hence, effluents from aquaculture have been used for garden applications or the production of hydroponic plants as a secondary treatment in the waste management procedure (Miller and Semmens, 2002). In some management practices, such as the study by Somboonchai and Chaibu (2013), four vegetables were grown in a hydroponic system integrated with catfish culture. However, the effluents from aquaculture such as shrimp farming contain not only nutrients but also other chemical substances such as antibiotics, herbicides and fungicides that potentially impact on the environment (Anantanasuwong, 2001). A review by Carvalho et al. (2014) indicated that pharmaceutical products, including antibiotics, hormones, analgesics and anti-inflammatory drugs, chemical compounds used for disinfection and cleaning, and endocrine-disrupting compounds can be assimilated by the plants. Therefore, while the potential for biomass production and nutrients recovery from wastewater are primary concerns in wastewater management systems (Turcios and Papenbrock, 2014), bioaccumulation of toxic substances is another aspect of concern. Additionally, increasing water scarcity in either dry regions of the world or in developing countries makes the reuse of wastewater in agriculture more important (Blumenthal et al., 2000). Nevertheless, it is of interest to identify whether or not a practice is productive and safe for both the environment and human health.

Several plant species can tolerate toxic substances by accumulating them in non-toxic forms or transforming them to either non-toxic or less toxic products. Most studies showed that textile dyes can be either adsorbed and accumulated or transformed to less or non-toxic substances by detoxifying enzymes, predominantly peroxidase, in plant cells (Govindwar and Kagalkar, 2010). The dye MG was found to be transformed to 4-dimethylamino-cyclohexa-2,4 dienone in *Blumea malcolmii* Hook. using enzyme laccase and the products had less toxicity toward *Phaseolous mungo* and *Triticum aestivum* when tested (Kagalkar et al., 2011). Rai et al. (2014) found that biodegradation of MG by *Aloe barbadensis* resulted in nontoxic metabolites, suggesting the possibility of using treated, dye wastewater for irrigation. Torbati (2016) reported that activity of antioxidative enzymes, namely SOD, POD and CAT, in *Spirodela polyrhiza* L. was increased with increased MG in the bathing medium. The activity of these enzymes allowed the species to tolerate MG at concentrations of 10 mg/L and 20 mg/L. However, knowledge on the degradation of synthetic dyes by vegetable plants is scarce since phytotransformation has been studied mainly in non-edible plants. Nowadays, the trend toward eco-friendly and sustainable production of any kind of product strongly influences consumers.

The current study investigated the application of wastewater containing MG from aquaculture for the production of pak choy (*Brassica chinensis* Tsen & Lee), a vegetable that is produced commercially in many Asian countries. It was hypothesized that being a member of the genus *Brassica* whose species usually have high antioxidant enzyme activity upon exposure to toxic substances (for example, Felicite et al., 2007; Song et al., 2009; Ma et al., 2013; Liu et al., 2014), *B. chinensis* may have ability to degrade MG dye and, hence, tolerate the dye at the low concentration used in aquaculture. If so, the reuse of water contaminated with MG could be applied. However, some *Brassica* species such as cabbage (*Brassica rapa* var. *pekinensis*) and Wisconsin fast plants (*Brassica rapa*) could take up and accumulate some toxic substances in their tissue, especially in the roots (Herklotz et al., 2010; Szczygłowska et al., 2011). Therefore, on the other hand, the MG dye in water may be accumulated in plant tissue and inhibit growth of the plant. Thus, the aims of this study were: 1) to study the effects of MG on the growth of *B. chinensis* and 2) to evaluate the accumulation of toxic substances in the edible parts of *B. chinensis*. The findings from this study will be useful for consideration in a wastewater management strategy, particularly for the reuse of aquaculture wastewater in crop irrigation.

Materials and methods

Plant materials

Seeds of pak choy (*Brassica chinensis* Tsen & Lee) were commercially obtained (Jet Plane Brand; Chia Tai Group Limited Company; Bangkok, Thailand) and germinated on a moistened sponge in the dark. When the seedlings were age 7 d, the nutrient mixed solution was applied replacing water, and the seedlings were allowed to grow under ambient conditions to age 14 d before being transferred to the growth medium used in the growth experiment.

Fourteen-day-old seedlings with 3–5 leaves and an average height of 8 cm were selected for the growth experiment. The seedlings were grown in nutrient mixed solution for 1 wk to allow for acclimation to the hydroponic growth conditions. The nutrient mixed solution was prepared from tap water and 1 ml/L of commercial A and B nutrient solution for hydroponic planting (Zen Hydroponics; Chiang Mai, Thailand). The pH and electrical conductivity (EC) of the nutrient mixed solution were monitored and maintained at 6.0–6.5 and 1.5–2.5 ms/cm, respectively.

Growth experiment

After 1 wk acclimation, 48 seedlings were distributed to four levels of MG concentration treatments: 0 mg/L (control), 1 mg/L, 2 mg/L and 4 mg/L ($n = 12$). The basal part of each seedling was fitted in a small plastic basket to hold the plant in an upright position and the baskets were fixed on the lids of 5 L plastic tub containers. One container with 12 seedlings was used for each treatment. The chemical formula of the MG used was $C_{23}H_{25}N_2Cl$ (analytical grade; Sigma-Aldrich; St Louis, MO, USA). The concentration of each treatment was obtained by adding the appropriate volume to make up 500 mg/L of MG stock solution to the nutrient mixed solution which hereafter is called the growth solution. The pH and EC of the growth solution were monitored and maintained as mentioned above and the growth solutions were renewed weekly. The experiment was maintained under ambient conditions with the air temperature 24–29 °C, relative humidity 41–60% and natural sunlight. Decolorization of MG in the growth solution at day 7 was detected spectrophotometrically using an ultraviolet–visible spectrophotometer (UV-1800; Shimadzu, Japan). The solution from each treatment (10 mL) was sampled and measured for absorbance at 400–800 nm compared with the absorbance of freshly prepared solution at the same concentration.

After 4 wk of growing, growth parameters (number of leaves, leaf length and width, shoot height and root length) were measured. Then, all plants were harvested and each plant was separated into root and shoot (leaves + stem) parts, and the shoots were stored at −70 °C in a freezer for further tissue analysis. The roots were abandoned since it is normally a non-used part of this vegetable and it was impossible to separate the plant roots from the supporting sponge. The weight of the shoot was measured after drying in a hot-air oven at 60 °C for 48 h and the final dry mass (DM) was determined. The experiment was conducted between February and March.

Tissue analysis

As the dry biomass of each individual plant sample (range 0.8–1.8 g) was not sufficient for measurement of all tissue content parameters, oven-dried plant materials from each treatment ($n = 12$) were pooled together and then cut into small pieces and mixed homogeneously before using in tissue analyses. The analyses of total N and oxalate were done in three replicates and since there were no differences among the groups of each treatment, the average of the three replicates was used for further analysis.

Total N and total oxalate contents

A plant sample (0.5 g) was used for analysis of the nitrogen content using the Kjeldahl method (Tennyson and Winlers, 2000) with slight modification. Each sample was digested with concentrated HCl prior to distillation. The NH_3(g) was trapped by boric acid during distillation. Finally, the amount of borate was quantified by titration with HCL. Then, the amount of HCL at the end point was used to calculate the nitrogen content. The total oxalate concentration was analyzed based on the method modified from Munro and Bassir (1969) using 1.0 g of dry plant material. Briefly, plant sample powder was extracted thrice by warming with 0.3N HCl (40–50 °C). Then the extract was precipitated with 5% $CaCl_2$ in acidic conditions. The precipitate was later re-dissolved in warmed 3N H_2SO_4 (70–80 °C). The solution obtained was further titrated with 0.01N $KMnO_4$ to determine the oxalate content. The analysis of plant tissue from each treatment was done in three replicates.

Attenuated total reflectance Fourier transform infrared spectroscopy analysis

The functional groups present in the MG dye structure and the functional groups potentially obtained from MG degradation in plant tissue were analyzed using attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) (Spectrum One model; Perkin-Elmer; Beaconsfield, UK). For the ATR-FTIR analysis, the samples were directly placed into the sample cell and the ATR-FTIR spectra of samples were scanned in the range 4000–650/cm.

Statistical analysis

Statistical analysis was performed using the SPSS version 22.0 software package (SPSS Inc.; Chicago, IL, USA). All data were tested for homogeneity of variance using Levene's test. Differences within groups were tested. Treatments effects on plant growth were determined by analysis of variance using type III sums of squares. Multiple comparisons of means at the 95% confident level were performed using Duncan's procedure.

Results

After 4 wk, plants grown in the solution with MG at a concentration of 2 mg/L or 4 mg/L had prominently stunted roots with blue-staining on the root surfaces. The depletion of the blue coloration in the solution was also observed and ultraviolet–visible spectrophotometric analysis (400–800 nm) of the growth solutions showed decreasing absorbance at 618 nm, which is the absorption maxima of MG, in all growth solutions after 1 wk of growth compared to freshly prepared solution (data not shown).

Effects of MG on plant growth

After 4 wk of growth, the size of the control plants and those grown at 1 mg/L MG were generally similar and significantly larger than the plants grown at 2 mg/L or 4 mg/L MG. Leaves of plants grown on solution without MG or with 1 mg/L MG were significantly longer and wider than those of the plants grown on solution added with MG at a concentration of 2 mg/L or 4 mg/L ($p < 0.001$). The roots of the plants grown at 2 mg/L or 4 mg/L MG were significantly shorter than those of the plants grown on solution without MG or with 1 mg/L MG ($p < 0.001$). The effects of MG on plant growth were also evident in the plant dry mass which was significantly reduced with increased MG concentrations in the growth solutions ($p < 0.001$) as shown in Table 1.

Tissue contents

The total N and oxalate contents in the plant shoots are presented in Table 2. Plants exposed to MG at all concentrations had a significantly higher total nitrogen content than plants in the control group ($p < 0.001$). However, the nitrogen contents were not different among the treatments with MG of 1 mg/L, 2 mg/L or 4 mg/L. The total oxalate content in the shoots of plants grown in nutrient solution with MG were significantly higher than that of plants grown without MG ($p < 0.001$).

Attenuated total reflectance Fourier transform infrared spectroscopy analysis

To compare functional groups present in MG and in plant tissues, ATR-FTIR analysis was performed in MG, in the plants grown without MG serving as a control and in the plants grown in MG-contaminated water at a concentration of 1 mg/L or 2 mg/L. The plants from the treatment with 1 mg/L were subjected to this analysis as their growth was nearly unaffected by the dye and the plants from treatment of 2 mg/L were selected as representative of the affected plants. The results are presented in Fig. 1 and Table 3.

The ATR-FTIR analysis of MG (Fig. 1A) resulted in peaks at 690/cm for the CH_2 stretch, a peak at 1445/cm for the C–H stretch, a peak at 1579/cm for the C=C stretch, a peak at 2810–2860/cm for the C–H stretch and a peak at 3403/cm for the O–H stretch; these results show the typical peaks of the MG used in this study. The ATR-FTIR spectra of the plants grown in solution with or without MG (Fig. 1B–D) showed the typical spectra of the plants which peaks at 1010–1097/cm for the C–OH bending, peaks at 1603–1618/cm for the C=C stretch, has a peak at 2916/cm for the C–H stretch and peaks at 3276–3283/cm for the O–H stretch. A prominent difference in the ATR-FTIR spectra between plant treatments was found in plants grown on 2 mg/L MG (Fig. 1D), where the peak at 670–690/cm occurred.

Discussion

The effects of MG on plant species have been mostly tested using seed germination and the plant seedling stage, with germination and seedling development being generally inhibited (for example, Kagalkar et al., 2011; Gopinathan et al., 2015). The current study found that MG contamination in water also caused negative effects to *B. chinensis*, particularly at concentrations greater than 1 mg/L. From the results, the negative effects of MG were strongly evidenced on root growth which was reduced by 50% upon exposure to MG of 2 mg/L and 4 mg/L compared to the control or 1 mg/L MG treatments. Similar effects on root growth were found in *Arabidopsis thaliana* grown on medium supplied with 4 mg/L of Crystal Violet and MG (Fu et al., 2013). The stunted roots may contribute to the overall reduction in plant growth since the uptake of water and nutrients could occur only via root transport under the hydroponic growth conditions used in this study. Nevertheless, the overall

Table 1
Plant height, leaf length and width, root length and dry mass (mean ± SD, $n = 12$) of *Brassica chinensis* grown at different concentrations of Malachite Green under hydroponic growth conditions and results of analysis of variance (*F*-ratio).

Growth parameter	Malachite Green concentration (mg/L)				*F*-ratio
	0	1	2	4	
Plant height (cm)	32.58 ± 2.74[a†]	31.00 ± 2.04[a]	28.54 ± 5.33[b]	27.25 ± 4.84[c]	4.03*
Leaf length (cm)	23.38 ± 3.33[a]	21.77 ± 2.18[a]	18.86 ± 2.84[b]	18.27 ± 3.15[b]	8.33***
Leaf width (cm)	7.21 ± 1.35[a]	6.73 ± 0.76[a]	5.91 ± 0.83[b]	5.17 ± 0.87[b]	9.99***
Root length (cm)	14.00 ± 1.77[a]	14.50 ± 3.91[a]	7.17 ± 1.52[b]	6.58 ± 0.94[b]	40.43***
Dry mass (g)	1.80 ± 0.73[a]	1.22 ± 0.48[b]	1.17 ± 0.27[b]	0.86 ± 0.17[b]	7.86***

† Different lowercase superscript letters indicate significant difference between treatments; * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Table 2
Total N and oxalate content in shoot tissue (mean ± SD) of *Brassica chinensis* grown at different concentrations of Malachite Green under hydroponic growth conditions and results of analysis of variance (*F*-ratio).

Tissue content (g/100 g dry matter)	Malachite Green concentration (mg/L)				*F*-ratio
	0	1	2	4	
Total N	4.28 ± 0.09[a]	4.90 ± 0.06[b]	4.85 ± 0.07[b]	4.85 ± 0.08[b]	46.22***
Oxalate	0.88 ± 0.09[a]	1.04 ± 0.05[b]	1.20 ± 0.07[c]	1.31 ± 0.05[c]	23.41***

†Different lowercase superscript letters indicate significant difference between treatments; *** = $p < 0.001$.

growth of *B. chinensis* in the current study indicated that the plant tolerates MG at a concentration of 1 mg/L.

MG has been suggested to be toxic to plants as it could be strongly absorbed on the surface of cellulose (Buvaneswari and Kannan, 2011) and taken up through the roots and accumulated in plant tissues (Fu et al., 2013). Saranya et al. (2011) found that the chlorophyll contents in *Hydrilla verticillata* decreased with increasing Basic Violet 14 dye concentrations from 5 mg/L to 25 mg/L, although the difference was not significant at 5 mg/L and 10 mg/L, and this result supported the inhibition of the dye on chlorophyll biosynthesis. In the current study, there was no evidence of chlorosis in the *B. chinensis* leaves at the concentrations applied (1–4 mg/L). However, the effect of MG on chlorophyll biosynthesis in this plant species should be better explained by pigment analysis.

Dye contamination in either the water or soil usually causes a reduction in the total content of macromolecules such as proteins and carbohydrates whilst it usually induces the activity of several enzymes used for dye degradation in exposed organisms. Triphenylmethane dyes such as Crystal Violet and Methyl Violet could cause lower protein synthesis which consequently inhibited cell growth in *Bacillus subtilis* (Ogawa et al., 1988). Jayanthy et al. (2014) found that soil contaminated with dyes from dyeing industries caused decreasing protein, total free amino acid and carbohydrate contents in *Vigna radiata*, whilst in the same plant, there were increases in the proline, glutathione and methyl glyoxal contents in either leaf or root tissue which indicated a response to abiotic stress. Moreover, the activities of lignin peroxidase, veratryl alcohol oxidase, laccase, tyrosinase and DCIP reductase were induced in *Aster amellus* Linn. and *Glandularia pulchella* (Sweet) Tronc. upon exposure to the dye Remazol Orange 3R (Kabra et al., 2011). According to those findings, there was an elevated nitrogen content in the shoot tissue of the plants, while plant growth was inhibited; in the current study the elevated level likely reflected an increase in stress responses upon exposure to MG.

Several plants species have potential for dye decolorization (Aubert and Schwitzguébel, 2004; Ghodake et al., 2009; Khandare et al., 2011; Rai et al., 2014). The decolorization of either the textile effluents or dye mixtures used can be achieved by adsorption and accumulation on plant surfaces, and mostly by phytotransformation or phytodegradation—the mechanism that degrades or transforms the dye into non-toxic products (Govindwar and Kagalkar, 2010). The degradation could be enhanced by rhizosphere-associated microorganisms, by enzymes excreted from or within roots (De Araujo et al., 2002: Davies et al., 2005) or even by enzyme extracted from leaves (for example, Carias et al., 2007) and cell cultures (for example, Kagalkar et al., 2011). In the current study, the adsorption of the MG dye to root surfaces, as could be seen by the blue staining, particularly in the treatments with MG of 2 mg/L or 4 mg/L, could be one mechanism that accounts for the depletion of MG dye in the growth solution that occurred in this study. According to Davies et al. (2005), adsorption of xenobiotics followed by its absorption, allows the binding of xenobiotics to plant roots. Retarded roots of *B. chinensis* growing with MG at 2 mg/L or 4 mg/L suggests toxicity of MG to plant roots. It has been reported that *B. juncea* has great potential for Reactive Red 2 degradation which is supported by the activities of the enzyme laccase and NADH-DCIP reductase predominantly present in roots (Ghodake et al., 2009). Nevertheless, Mukherjee and Das (2014) reported that the decolorized level of MG by *Enterobacter asburiae* Strain XJUHX-4TM decreased as the exposure time and concentration of the dye increased due to the toxicity of the dye to bacterial cells. Detoxification of MG may be achieved through some metabolic activities present in the roots. However, the 28 d of exposure used in the current study and at higher concentrations (2 mg/L or 4 mg/L) may have caused a reduction in the detoxification ability of the plant and resulted in plant toxicity.

The results obtained from the FTIR analysis can be used as a tool to predict the changes in the functional groups of the original dye molecules (Govindwar and Kagalkar, 2010). The ATR-FTIR analysis was performed in the study to detect whether or not there were functional groups possibly obtained from MG that had accumulated in the edible plant part. The ATR-FTIR spectra comparison between plant samples from the control and treatment groups suggested that at the concentration of 1 mg/L, MG may be transformed before either being taken up by the plants or translocated into the shoot tissue. Kagalkar et al. (2011) showed that *Blumea malcolmii* Hook. could degrade MG dye and the degradation gave 4-dimethylamino-cyclo-hexa-2,4 dienone as the transformed product. Fu et al. (2013) found that transgenic *Arabidopsis* converted Crystal Violet to Lleucocrystal Violet (LCV), which is non-toxic to the plant, and LCV was then gradually degraded by other endogenous enzyme activities. The products obtained from the phytotransformation of MG were usually non-toxic to tested plant species in all phytotoxicity reports

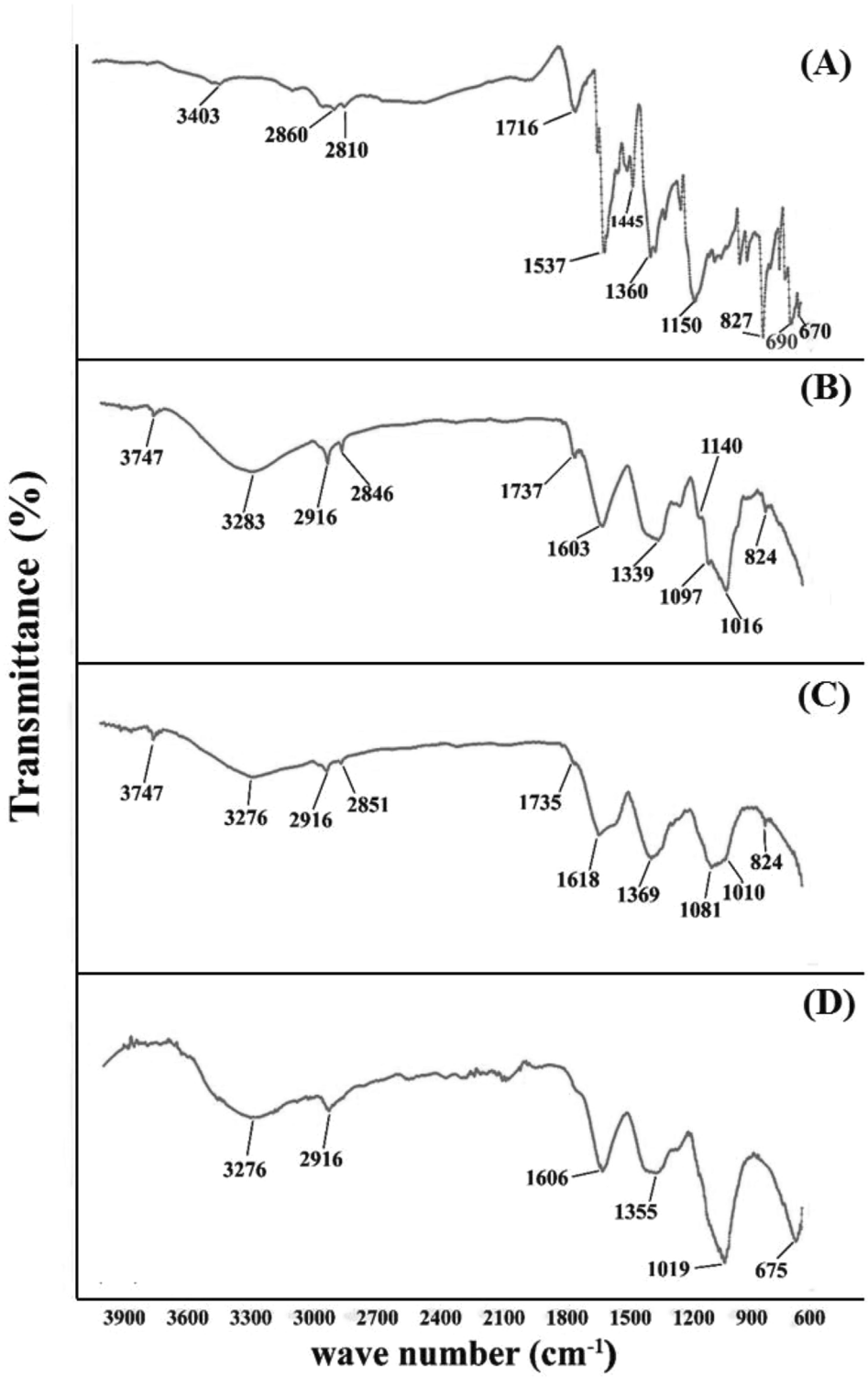

Fig. 1. Spectra of Malachite Green (MG) using attenuated total reflectance Fourier transform infrared spectroscopy with plant tissue from each treatment: (A) MG; (B) plant grown with 0 mg/L MG (control); (C) plant grown with 1 mg/L MG; (D) plant grown with 2 mg/L MG.

(for example, Kagalkar et al., 2011; Rai et al., 2014). Hence, this might explain the unaffected growth of *B. chinensis* in the growth medium with a concentration of 1 mg/L MG, whilst the ATR-FTIR spectra (with the peak at 670–690/cm) suggest that there was a similar functional group in MG and in the shoot of plants grown at 2 mg/L MG. Although it might be possible that the functional group originated from MG or could have been obtained from MG degradation, it could also be the substance synthesized by the plant in response to MG. Hence, identification is still needed of the substances, using techniques such as high performance liquid chromatography mass spectrometry (Fu et al., 2013). Together with this result, the effects on root growth at MG concentrations of 2 mg/L

Table 3
Presence of Malachite Green (MG) spectra from Fourier transform infrared spectroscopy in control plants (0 mg/L MG) and plants treated with 1 mg/L and 2 mg/L MG.

Vibration	Wave number (/cm)	MG	Plant + MG (mg/L)		
			0	1	2
N-H stretch	3,747	–	✓	✓	–
O-H stretch	3,403	✓	–	–	–
	3,276–3,283	–	✓	✓	✓
C-H stretch	2,916	–	✓	✓	✓
	2,810–2,860	✓	–	–	–
	2,846–2,851	–	✓	✓	–
C=O stretch	1,735–1,737	–	✓	✓	–
	1,716	✓	–	–	–
C=C stretch	1,603–1,618	–	✓	✓	✓
	1,579	✓	–	–	–
C-H stretch	1,445	✓	–	–	–
C-N stretch	1,339–1,360	✓	✓	✓	✓
	1,150	✓	–	–	–
	1,140	–	✓	–	–
C-OH bending	1,010–1,097	–	✓	✓	✓
NH_2 stretch	824–827	✓	✓	✓	–
CH_2 stretch	670–690	✓	–	–	✓

and above suggested that these concentrations are toxic to root cells and may result in the accumulation of toxic substances in the shoot tissue of *B. chinensis*. The increased oxalate content in the shoot tissues of plants exposed to MG in the current study might be accounted for by an enhanced tolerance mechanism in the plant, as Nilratnisakorn et al. (2008) suggested that the precipitation of metal-dye complexes in leaves and roots as calcium oxalate, calcium silicate and silica in *Typha angustifolia* Linn. (Narrow-leaved Cattail) is the mechanism that avoids damage to plant cells.

With regard to the potential health risk of some bio-accumulated substances in food products, the accumulation of possibly toxic derivatives obtained from MG transformation such as Leucomalachite Green (Srivastava et al., 2004) in plant tissues still needs to be identified. In addition, as oxalate comprises 75% of kidney stones (Hesse and Siener, 1997) and consumption of high oxalate foods can promote the risk of kidney stone formation (Holmes and Assimos, 2004) in the human urinary tract, *B. chinensis* grown with MG contaminated water in this study, having increased the oxalate content, may pose a risk of kidney stone formation as well.

The results of plant growth revealed that *B. chinensis* was able to grow in water contaminated with MG at a concentration of 1 mg/L and had the ability to remove the dye from contaminated water through adsorption via its root surface. The tissue contents, total N and total oxalate concentrations, and the ATR-FTIR spectra analyzed in the current study indicated that the tolerance of the plant to low levels of MG could be achieved by increasing stress responses and the accumulation of toxic substances in oxalate form, which hamper toxicity to plant cells. However, the plant could not tolerate high concentrations (2 mg/L or 4 mg/L in this study) of MG resulting in the increased accumulation of toxic substances in plant tissue and the reduction of overall growth as a consequence. A conclusion from the current study is that although the integration of hydroponic plant production for wastewater management in aquaculture that is still using the dye at a low concentration can be applied without noticeable phytotoxicity symptoms, this might pose a potential health risk for humans. Hence, the detection and identification of substances accumulated in plant tissue is still needed.

Conflict of interest

There is no conflict of interest.

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