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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.