Effects of Malachite Green Contaminated Water on Production of Pak Choy and Chinese Convolvulus

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***Abstract*—Malachite green is used in industries and aquaculture and disposed in the effluents. In this study, effects of malachite green on growth of *Brassica chinensis* and *Ipomoea aquatica* were studied in order to evaluate possibility of using dye-contaminated wastewater for irrigation. Seedlings of the plants were grown in growing material and watered with tap water containing malachite green at the concentrations of 0 (control), 1, 2, 10, and 20 mg/L for 21 days. At harvest, number of leaf and shoot and root dry weight of all plants were measured. For both species, biomass values of treated plants were similar to the control (dry weight were 0.6-1.0 and 1.1-1.7 g/plant for *B. chinensis* and *I. aquatica*, respectively) and *B. chinensis* was more sensitive to contaminant compared to *I. aquatica*. There was no sign of MG and leucomalachite green detected in root and shoot tissues of plants treated with MG at 20 mg/L, tested by TLC. After plant harvest, toxicity of the growing material was tested on mung beans. Percent germination (83-97%), seedling fresh weight (0.3-0.5 g/plant), and shoot length (11-12.5 cm) were similar to the control indicating that contaminant in growing material did not pose detrimental effect on mung beans. Based on these results, the water contaminated with low concentration of MG may serve as fertirrigation water to compensate water shortage.**

***Index Terms*—*Brassica*, fertirrigation, *Ipomoea*, triphenylmethane dye, wastewater reuse**

I. Introduction

Increased human population has been the main cause for increasing water demand due to increase of industrialization and expansion of irrigated agriculture for at least a decade. Addition to this, heavily polluted freshwater pushes water stress situation even more severely, particularly in developing regions such as Africa and Asia where effective strategy for water resource management is limited [1]. The water stress situation not only leads to sanitation problem but also food security problem as it can cause reduction in food production [2]. Hence, attempt on reusing industrial wastewater for agricultural purpose has been emerged a few decades, especially in developing countries [3].

Effects of textile wastewater on crops have been widely reported and the effects differ among species of plant and types of contaminates. Most of findings explained adverse effects of untreated textile effluents on seed germination and plant growth at early growing stage, and those effects diminished when using treated wastewater or diluted effluents [4]. Heavy metals and salinity usually be the concerned physico-chemical parameters of textile wastewater that are toxic to plants [5]. Improperly treated wastewater also still contained metals and other toxicants that potentially affected plant growth [6]. Reference [7] found that chemically treated wastewater was not completely improved in quality and still toxic to *Brassica nigra* and *Cyamopsis tetragonolobus*. Toxicity of dyes stuff in wastewater has come to focus since many dyes have been proved as a carcinogenic substances and pose detrimental effects on environment. However, our knowledges on dyes hazardous is still incomplete and conventional treatments such as chemical treatment inefficiently eliminate toxic nature of the dyes [8]. Detrimental effects of the dye such as Malachite green (MG) on soil organisms including microbes and earthworm was still lately elucidated [9]. Moreover, reports on assessment on plant poisoning is still scarce.

In Thailand, textile and garment manufacture has been expanding among other manufacturing sectors [10]. In addition, small scale and local manufacturers, such as community-based manufacturer, have been promoted to overcome economic crisis occurs among poorer rural population in Thailand for a decade. Industrial wastewater need to be treated to meet discharge limits for BOD, COD, pH, and some other heavy metals before discharge following the water quality standard used by the pollution control department [11]. However, as legislation controlling small scale manufacturer as well as discharge limit for dyes are lacking, local manufacturer usually discharges untreated waste water directly to soil or nearby water body. The discharge of textile effluent potentially causes surrounding environment contaminated with toxic dyes and chemicals [3], [5]. The polluted soil and water might consequently cause adverse effects, in term of food safety and security, to human populated in rural area where the food production are involved in their

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backyard gardens or from small-scale farming [10]. In addition, the polluted water can be unintentionally used for agriculture [12].

Aims of this study were 1) to assess growth responses of two plant species to dye contaminated in irrigated water and 2) to assess the accumulation of dye derivative(s) in the plants tissue. The two plant species used in the study were Pak choy (*Brassica chinensis*) and Chinese convolvulus (*Ipomoea aquatica*). The dye subjected to the study was Malachite Green (MG) which is widely used in textile industries and also used in aquaculture [13] and the concentrations used were 1, 2, 10, and 20 mg/L. The first two concentration levels were assigned to cover the concentration used in aquaculture and the latter two were assigned following the concentration limited, in the form of BOD, in effluents to be discharged to receiving water [11]. Irrigation practice was conducted after the plants were grown for four weeks.

II. Materials and Methods

A. Plant Preparation

Seeds of Pak choy (*Brassica chinensis* Tsen & Lee) and Chinese convolvulus (*Ipomoea aquatica* Forsk. var. *reptan*) (Chia Tai Group) were commercially purchased. Seeds were germinated by sowing them in growing material filled in germination container and watering to moist. When seedlings reached two to three leaves stage, three seedlings were transferred to a plastic pot containing 1.4 kg of growing material. The growing material used in this study was the commercial brand “Din Phu Wiang” consisting of soil, coconut fiber, and compost. Seedlings were watered with tap water and nourished with fertilizer (N:P:K=15:15:15) for four weeks before starting irrigation experiment.

B. Experimental Setup

For each plant species, three pots were used as control which was watered by tap water. Others were distributed to treatment conditions which were watered by water contaminated with 1, 2, 10, and 20 mg/L of MG (three replicates). Watering with 200 ml of water or MG contaminated water was applied at a two-day interval for the first week and then everyday afterward, accounting for 21 days in total.

C. Data Collection

Plants with similar size to those subjected to the experiment were harvested to determine initial fresh weight and dry weight. Number of leaves and plant dry weight were measured both before starting the experiment and at harvest.

D. Detection for Malachite Green and Leucomalachyte Green (LMG)

Possibility to accumulate MG and LMG in plant tissues was preliminary tested by thin layer chromatography (TLC) following the method explained in Ref. [14]. Dried shoot and root samples of both vegetables from the control and the treatment of 20 mg/L were ground with homogenizer, then 0.5 g of each sample was extracted by 20 ml of ethyl acetate for 24 hours. The extracts were evaporated to dryness and the extracted powders were subsequently diluted with methanol. Presence of MG and LMG in the extracts was examined by TLC using silica gel. The solvent system was n-propanol: ethyl acetate: acetic acid: distilled water (6:1:1:2 v/v) and the visualized sign of each substance was done both under UV chamber and in iodine chamber.

E. Toxicity Test on Contaminated Soil

Toxicity of soil (growing material) after the plants were harvested was tested on mung beans seed germination and seedling growth. Ten seeds were saw in the soil from each treatment (three replicates) and water to moist for one week, and then number of seedlings, fresh weight of seedlings, and shoot length were determined. Percentage of seed germination was then calculated by using (1), and all parameters were expressed by their average values.

$$\text{Germination (\%)} = (\text{no. seedlings/no. seeds}) \times 100 \quad (1)$$

F. Statistics

Growth parameters were analyzed by one-way analysis of variance (ANOVA) using Duncan’s test to clarify differences between groups. Statistic program used in the analysis was IBM SPSS Statistics 19.

III. Results

A. MG Application

The quantity of MG used in the irrigation experiment totally accounted for 4.2, 8.4, 42, and 82 mg/kg of soil in the treatment of 1, 2, 10, and 20 mg/L, respectively.

B. Plant Growth

Considering plant dry mass, either leaf or root, and number of leaves that each plant produced (Table I and Table II), the two vegetables could grow well when being irrigated with MG contaminated water. Although the production, by mean of biomass, of both vegetables was not affected, we found that *B. chinensis* grown under the treatments of MG at 2, 10, and 20 mg/L produced lower number of new leaves (1.66 leaves on average) compared to the plants grown under the control and the treatments of 1 mg/L (3 leaves on average). In addition, leaves of plants from the treatments of 2, 10, and 20 mg/L were usually smaller and shorter than those of plants from the control and the treatment of 1 mg/L treatment (data not show). The morphological difference in *I. aquatica* was, however, not found.

TABLE I. Growth Parameters of *Brassica Chinensis* Grown on Soil Irrigated with MG Contaminated Water at Different Concentrations (0, 1, 2, 10, and 20 mg/L). Data Presented as Mean ± S.D., *N* = 3.

treatment concentration (mg/L)	number of leaves/plant	shoot dry weight (g)	root dry weight (g)
0	7.89±0.38	0.51±0.15	0.12±0.03
1	7.44±0.77	0.63±0.16	0.12±0.04
2	6.22±0.69	0.52±0.23	0.11±0.06
10	7.00±0.58	0.53±0.36	0.09±0.03
20	7.33±0.88	0.82±0.34	0.18±0.04

TABLE II. GROWTH PARAMETERS OF *IPOMOEA AQUATICA* GROWN ON SOIL IRRIGATED WITH MG CONTAMINATED WATER AT DIFFERENT CONCENTRATIONS (0, 1, 2, 10, AND 20 MG/L). DATA PRESENTED AS MEAN ± S.D., *N* = 3.

treatment concentration (mg/L)	number of leaves/plant	shoot dry weight (g)	root dry weight (g)
0	10.67±5.03	1.04±0.20	0.63±0.12
1	8.89±3.60	0.65±0.16	0.57±0.08
2	13.89±4.00	0.83±0.15	0.72±0.10
10	9.22±1.50	0.71±0.17	0.43±0.11
20	12.44±1.71	0.91±0.19	0.53±0.10

C. Detection of MG and LMG

The visualization of MG could be seen as the blue spot under UV light and that of LMG could be seen as the greenish-blue spot after incubated in iodine chamber (Fig. 1). Our result showed that the visualization of extracts was different from MG and LMG, and did not differ between control and treatment samples. Therefore, by mean of this detection method, we neither found sign of MG nor LMG accumulated in plant tissues.

D. Toxicity Test on Contaminated Soil

The data presented in Table III show that soil (growing material) used in the experiment did not pose any adverse effect on mung bean seedlings. Germination of seeds on contaminated soil was as good as control and the germination percentages were in the range of 80 to 97%. Fresh weight and shoot length of seedlings were also not different among treatments ($p>0.05$).

IV. DISCUSSION

Responses of plants to synthetic dyes varies depending on plant species, types and concentrations of the dyes, and growing conditions. Studies on effects of MG on crop plants have been performed mostly on seed germination and early seedling stage. Ayed *et al*. [15] found that concentration of MG at 100 mg/L reduced seed germination and length of plumule and radical of *Triticum aestivum* and *Sorghum bicolor* tested on filter paper. While Gopinathan *et al*. [9], also conducted the filter paper test, reported that MG at the concentration from 10 to 100 mg/L do not affect germination of *Vigna radiata* (Mung beans), *Brassica nigra* (Mustard), and *Triticum aestivum* (Wheat). For seedlings of *Arabidopsis* grown in agar plate, the concentration of MG at 4 mg/L could severely reduce growth of their roots, shoots, and leaves [12]. Plants at maturation stage respond to MG by reduction of photosynthetic pigments and increase of antioxidant activities. The concentration of MG at 20 mg/L could reduce chlorophyll *a*, chlorophyll *b*, and total carotenoids contents in *Spirodela polyrhiza* after 7 days of exposer to contaminant [16]. In this study, the toxicity symptoms such as acute wilting, chlorosis, reduced germination, and biomass reduction were not observed. Based on the results of plant growth, it could be implied that the quantity of MG used in this study which were 4.2, 8.4, 42, and 82 mg/kg of soil in the treatments of 1, 2, 10, and 20 mg/L, respectively, did not pose adverse effect on growth of *I. aquatic* and *B. chinensis*.

Figure 1. Results from thin-layer chromatography (TLC) after incubating in iodine chamber: 1 = root of controlled Pak choy, 2 = root of controlled Chinese convovulus, 3 = root of Pak choy grown with MG at 20 mg/L, 4 = root of Chinese convovulus grown with MG at 20 mg/L, 5 = shoot of controlled Pak choy, 6 = shoot of controlled Chinese convovulus, 7 = shoot of Pak choy grown with MG at 20 mg/L, 8 = shoot of Chinese convovulus grown at 20 mg/L, 9 = malachite green (MG) and 10 = leucomalachite green (LMG)

TABLE III. RESULTS FROM SOIL TOXICITY TESTED ON MUNG BEAN SEEDLINGS. DATA PRESENTED AS MEAN ± S.D., *N* = 3.

treatment concentration (mg/L)	germination (%)	seedling fresh weight (g)	shoot length (cm)
0	83.33±15.28	0.43±0.04	11.14±1.09
1	86.66±5.77	0.45±0.04	11.96±0.59
2	96.67±5.77	0.47±0.02	12.11±0.39
10	86.67±5.77	0.49±0.01	12.12±1.41
20	90.00±10.00	0.38±0.01	11.27±1.19

As plants mainly take up nutrients in the soluble forms through their roots, hydroponic culture systems provide certain chemical elements more readily for uptake by roots compare to soil culture systems [17]. Our previous study showed that biomass of *B. chinensis* was significantly reduced when supplied with MG-contaminated nutrient solution at 2 mg/L for four weeks in hydroponic culture system (unpublished data). In the present study, although the production of new leaves and size of leaves of *B. chinensis* were reduced at the concentrations from 2-20 mg/L, effect on biomass of both *B. chinensis* and *I. aquatica* was not observed even at the highest concentration provided (20 mg/L). According to Buvaneswari and Kannan [18], MG could be rapidly adsorbed on cellulose surface by chemisorption mechanism which makes it poorly recovered, and the adsorption was increased with increased absorbent dosage. The MG dye applied in this experiment might be adsorbed on growing material surface, such as coconut fiber which mainly composes of cellulose, and poorly released in soluble form that can be later reach to plant roots. Hence, toxicity of the dye to overall growth of *B. chinensis* and *I. aquatica* and to germination of mung bean were not observed in this study.

Several plant species have potential to degrade dye which consequently reduce toxicity of the dyes. Activity of the enzymes superoxide dismutase, peroxidase, and catalase in roots of *Nasturtium officinale* R. Br. played important roles in the dye degradation and resistance of the plant to the dye BR46 [19]. *Salvinia molesta* could degrade the dye Rubine GFL using oxido-reductive enzymes in stem tissue making the plant tolerate to the dye, and giving non-toxic degradation products [20]. Degradation of MG has been reported mainly in microorganisms such as *Kocuria rosea* MTCC 1532 [14], *Staphylococcus epidermidis* [15]. Recently, some plant species have been studied and reported to have ability to degrade MG such as *Blumea malcolmii* Hook. [21] and *Spirodela polyrhiza* [16]. The peroxidase enzyme extracted from leaves of *Ipomoea palmata* was able to degrade triphenylmethane dye [22]. In the present study, unaffected growth of *I. aquatica* suggests that, in addition to low concentration of toxic substance remaining on growing media surface, the species might possess an ability to degrade MG which partly reduces toxicity of the dye and makes it more tolerant to the dye than *B. chinensis*.

Malachite green that has been taken up by catfish can be rapidly metabolized to leucomalachite green, which was suggested to be the target for monitoring [23]. Considering that the report on accumulation of leuco-form of MG in plant tissue is rare, however, in *Arabidopsis thaliana*, leuco-form of crystalviolet, another triphenylmethane dye, was detected in the transgenic plant exposed to the dye and this leuco-form of the dye is non-toxic to the plant itself [12]. In the present study, leucomalachite green was not detected by using the TLC technique. According to Fu et al. [12], leuco-form of MG might be further degraded by enzyme activity in plant cells or might be accumulated in the form that cannot be extracted by the technique used in the present study. Hence, determination of the dye and its derivatives in plant tissue is still need to be studied using various reliable methods. Together with this, antioxidative enzyme activity in vegetable plants is the future prospective needed to be studied in order to confirm the occurrence of phytodegradation process in the species.

V. Conclusions

Based on our findings, we conclude that use of MG contaminated water at a low concentration for irrigation on the first crop do not cause adverse effect on production of *B. chinensis* and *I. aquatic* and the contaminated soil did not toxic to plant that might be raised afterward. However, different plants respond differently to the contaminant as *B. chinensis* seem to be more sensitive than *I. aquatica*. The use of adsorbent as a mixture of growing materials could also reduce the toxicity of contaminants by adsorption of the contaminants on its surface. Hence, in case of necessary, the water contaminated with low concentration of malachite green such as treated wastewater or discharge from aquaculture may serve as fertirrigation water to compensate water shortage. However, for sustainability of food production, the study on effects of long-term application on plant growth and biochemical responses, and accumulation of toxic substances in either growing media or plants tissue is still required.

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