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Effect of irrigation with microcystins-contaminated water on growth and fruit quality of *Cucumis sativus* L. and the health risk

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ABSTRACT

Microcystins (MCs) are released into the lake during the outbreak of cyanobacteria and could enter the farmland ecosystem by irrigating. This study investigated the effect of MCs on growth, yield and fruit quality of cucumber and evaluated the potential risk of MCs. Cucumber plants were irrigated daily for 7 days with MCs extraction contaminated water (0, 1, 10, 100, 1000 μg/l), and then, cultivated with uncontaminated water to final harvest. Results show that 10 μg/l MCs exposure inhibited the growth of cucumber at different growth stages, and the order of decreased degree in the growth of cucumber was seedling stage > early flowering stage > fruiting stage. Contents of vitamin C, soluble sugar and organic acid in fruits of cucumber exposed to10 μg/l MCs at seedling stage were decreased. MCs at concentrations of 100 μg/l and 1000 μg/l significantly decreased the growth of cucumber at different growth stages, and reduced cucumber yield at fruiting stage. There were even no fruits at seedling stage and early flowering stage. The estimated daily intake of MCs in fruits exposed to 100 μg/l and 1000 μg/l MCs at fruiting stage were 0.103 μg/kg and 0.198 μg/kg, exceeding WHO limit. It indicates that human should exercise care when ingesting cucumber fruit as a part of their diet and strengthen agricultural irrigation management to prevent cucumbers from irrigating with MCs contaminated water.

1. Introduction

Toxic cyanobacterial blooms have become increasingly widespread in aquatic ecosystems, potentially as a consequence of eutrophication and climate change (Luerling et al., 2017). Under such condition, hepatotoxic cyanotoxins are produced by several cyanobacteria genera such as Microcystis, Anabaena, Plankthotrix, Nostoc (Li et al., 2017). They are cyclic heptapeptides of which around 80 variants have been identified (Gurbuz et al., 2009). Microcystins (MCs) are the most widely investigated cyanotoxins because of their frequency in nature and their adverse health effects on humans (Sviržev et al., 2017), as well as fish, birds, zooplankton, aquatic and terrestrial plants and mammals (Corbel et al., 2014).

MCs can be found mainly inside the producer cells (75%) but also dissolved in the aqueous media at concentrations dependent on cyanobacterial decay (Zurawell et al., 2005). Although most of cyanobacterial blooms occur in open aquatic systems such as oceans, rivers, lakes, ponds, *etc.*, they can also appear in waters intended for plant irrigation and agriculture. The impact of MCs on agroecosystems is also concerned when irrigation water is contaminated with MCs. MCs' main molecular targets are protein phosphatases PP1 and PP2A that are involved in several physiological and molecular processes (Mackintosh et al., 1990). Irrigation water contaminated with MCs also can inhibit seed germination and plant growth (Chen et al., 2004; Saqrane et al., 2008), plant production (El et al., 2012; Saqrane et al., 2009), decrease photosynthetic activity and induce oxidative stress (Bittencourt-Oliveira et al., 2016; El et al., 2011; Lahrouni et al., 2013). In addition, MCs can also act as tumor promoters, MCs can be absorbed and accumulated in plant tissues (Chen et al., 2010; Gutiérrez-Praena et al., 2014), and become a potentially dangerous to human health if people consume edible parts of crops and vegetables irrigated with MCs contaminated water (Bittencourt-Oliveira et al., 2016). Currently, the quality and safety of crops irrigated by MCs contaminated water are one of the most concerned issues. Machado et al. (2016) found that the content of ascorbic acid is significantly decreased by 10 and 50 μg/l in of carrot roots. The content of protein and amylose in grains of rice exposed to 100 μg/l MCs are lower than the control (Liang et al., 2016). It is worth noting that response of plants to MCs is relevant with exposure time, MCs concentrations, plant species and stages, and experimental conditions (laboratory or farmland). In the reality, the

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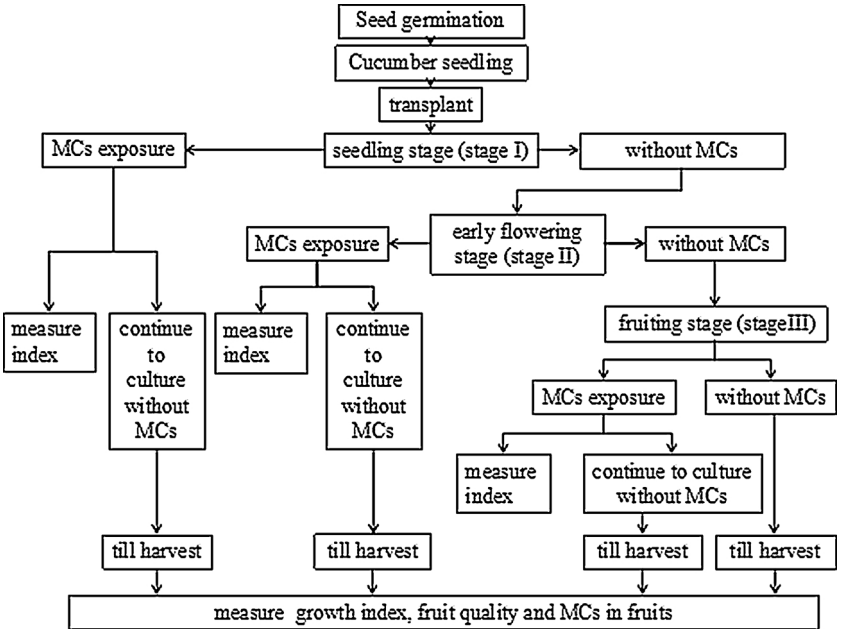

Fig. 1. Experimental design flow chart.

exposure of plants to MCs is a discontinuous process where plants could be exposed to MCs at different growth stages and followed by a restoration period without MCs exposure, or with decreased severity of exposure. Therefore, we established a novel experimental model that the experimental plants at different growth stages were first exposed to microcystins-contaminated water for 7 days and then underwent a recovery period without exposure of MCs till harvest. Based on this setting, it can be interesting to clarify the toxic effects of MCs on crops and their fruits. It also can be informative to evaluate the potential harm of MCs to human health by taking MCs contaminated fruits as a part of the diet.

Cucumber is a popular vegetable widely cultured around the world. Cucumber contains protein, carbohydrates, vitamins, crude fibers, and other nutrients which humans need. So, cucumber is one of the most commonly popular vegetables with high consumption. In the present study, we studied effects of irrigation with microcystins-contaminated water on growth when they are exposed to MCs for 7 days, and after harvesting cucumber fruits, we studied the effects MCs on yield and quality of fruits of cucumber as well as accumulation of MCs in fruits when they restored to harvest, a condition that is closer to the real situation when MCs contaminated water is used to irrigate. Finally, we evaluated the human health risk by comparing the estimated daily intake (EDI) of cucumber fruits treated with MCs contaminated water in our experiments and Tolerable Daily Intake (TDI) established by WHO to figure out whether the MCs content exceeded the international standard and would lead to potential health risk. These results will help us to scientifically evaluate the impact of MCs on crop plants and provide basic data for the rational irrigation of crops.

2. Materials and methods

2.1. Extraction and determination of MCs

MCs used in our experiments were extracted from fresh cyanobacteria in Taihu Lake (30°56′–31°34′N, 119°54′–120°36′ E, the third largest freshwater lake in China), According to the method described by (Liang et al., 2016) and slightly modified. Briefly, about 1 g of lyophilized cyanobacteria cells was added to 5% glacial acetic acid (v/v) for extracting. The homogenates were extracted for 2 h and centrifuged at 8000*g* at 4 °C for10 min. Then, the supernatant was collected and the residue re-extracted two times as before. All supernatants were combined together, and subjected to Sep-Pak C18 cartridges (Waters Corporation, Milford, MA) preconditioned with 10 mL methanol (100%) and 10 mL ultrapure water. After that, the loaded column was washed with 20% methanol, and the cartridges were then eluted using 100% methanol with 0.1% trifluoroacetic acid (TFA). The MCs fraction was evaporated by rotary evaporation at 40 °C and then dissolved in 1 mL ultrapure water. The concentration of total MCs was measured by ELISA (Microcystins plate kit, Beacon Analytical Systems Inc., Saco, ME).

2.2. Plant culture and MCs exposure

Cucumber (*Cucumis sativus* L.) seeds of "Xin jin yan 4" were supplied by Yang Gao Seed Company (Shanxi, China). The sterilization and germination of cucumber seeds were carried out as described in previously reported methods (Liang and Wang, 2015) and slightly modified. Briefly, $HgCl_2$ (0.1%, w/v) disinfected seeds, and then washed with deionized water 3 times. After soaking in distilled water for 8 h, the cucumber seeds were cultured for germination in Petri dishes with a layer of filter paper in an incubator (25 °C). After 6 days, the germinated cucumber seeds were moved to the 6.88 L turnover box with vermiculite. When 2 pieces of true leaves appeared, cucumber seedlings were cultured with the routine nutrient solution for hydroponics. Cucumber seedlings were cultured with Hoagland nutrient (Jiang et al., 2011) in a chamber with a 16-h photoperiod (200 μ mol $m^{-2} s^{-1}$) at the average temperature 27 ± 1 °C during the day and 20 ± 1 °C at night, the relative humidity between 60 and 70%. The nutrition solution was renewed every 3 days.

The experimental design for this study was shown in Fig. 1. When the third leaf of cucumber developed completely (seedling stage, stage I), the cucumber plants were treated daily with MCs crude extract at different concentrations for 7 days. When the sixth leaf of cucumber developed completely (early flowering stage, stage II), the cucumber plants were treated daily with MCs crude extract at different concentrations for 7 days. When the eleventh leaf of cucumber developed completely (fruiting stage, stage III), the cucumber plants were treated daily with MCs crude extract at different concentrations for 7 days. After a 7-day exposure, half of the cucumber at three stages (stage I, II and III) were taken to measure the growth index and the MCs content in roots and the rest were cultured under the control conditions (without