Effect of Microcystin on Growth And Development of Some Agricultural Plants Seedlings

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Abstract

A marked correlation between the abundance of *M. aeruginosa* blooms and high temperature, pH, N and P content of the pond water was observed during studies. Extract of *M. aeruginosa* showed toxicity to mice and showed the absorption maximum at 236 nm. Co-chromatography and HPLC analysis using standard microcystin showed that the compound present in MT-2 is identical to standard microcystin-LR. Intraperitoneal administration of crude and purified toxins of *M. aeruginosa* caused death of mice after 40-45 min. Microcystin content in water was also determined and revealed the presence of microcystin-LR in both the ponds Laxmikund and Durgakund respectively. The impact of partially purified microcystin was studied on plants system. Germination percent, growth and antioxidative activity of four plants (rice, cauliflower, mustard and onion). A drastic inhibition in percent germination was observed in mustard, cauliflower and onion seeds was observed in the presence of 2.5µg/ml microcystin. However, there was no significant effect of this concentration of MC on seed germination in rice. Growth (evaluated in terms of shoot and root length, fresh weight and total chlorophyll content) of all four plant seedlings was also significantly affected at the same concentration of microcystin. During antioxidative studies, significant increase in activity of SOD, POD and CAT was observed in the presence of different concentrations of microcystin in seedlings of all plants tested.

Keywords: Microcystins, cyanobacteria, seed germination, rice, antioxidant enzymes

Introduction

Besides showing animal toxicity, microcystins MCs are also known to cause various adverse effects on plants such as inhibition of growth, photosynthesis and other metabolic processes (Wiegand and Pflugmacher 2005; Saqrane et al. 2008; Saqrane and Oudra 2011; Zhang et al 2020; Campos et al 2021). Kós et al. (1995) for the first time reported the growth inhibition of white mustard (*Sinapis alba*) seedlings by MCs and crude extracts of MC containing cyanobacteria. Crop irrigation with water containing toxic cyanobacterial cells or MCs may produce serious health hazard to human health due to consumption of plants containing MC (Buratti et al 2017). Codd et al. (1999) showed the persistence of MC-LR and toxic *M. aeruginosa* cells on the leaves of salad lettuce (*Lactuca sativa*) after spray irrigation with water containing toxic cyanobacteria. Since then effect of MC contaminated water on several plants has been studied by several workers with traditional model organisms such as alfalfa, mustard, beans, tobacco, potato and rice and the uptake of the toxin and its effect on parameters such as growth inhibition and impairment, occurrence of chlorosis and necrosis, inhibition of photosynthesis, inhibition of single-strand DNAse activity and anthocyanin accumulation has been demonstrated (McElhiney et al. 2001; Hamvas et al. 2003; Gehringer et al. 2003; Chen et al. 2004; Saqrane et al. 2008). Accumulation of sufficient concentration of MC by terrestrial plants has been shown by several workers (Kurki-Helasmo and Meriluoto 1998; Yin et al. 2005b; Järvenpää et al. 2007). It has been shown that MCs are able to induce oxidative stress in plants and MC contaminated water could be one of the abiotic stresses affecting plant productivity (Chen et al. 2004; Pereira et al. 2009; Bibo et al. 2008; Chen et al. 2010; Campos et al 2021; Yadav et al 2022). Changes in activities of antioxidative system including superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) has been observed in plants due to exposure to MC (Chen et al. 2004; Yin et al. 2005a; Chen et al. 2010). Significant increase in glutathione S-transferase and glutathione peroxidase activities has also been observed in *Lepidium sativum* with varying toxin levels (Gehringer et al. 2003; Stüven and Pflugmacher 2007). Studies with animal systems have indicated that oxidative stress may play a significant role in the pathogenesis of MCs toxicity in animals and humans (Zegura et al. 2003; Dittmann and Wiegand 2006; Amado and Monserrat 2010) but very little is known about their potential to produce such effects in plant system. In the present paper we have studied the effect of MC on seeds germination and growth of seedlings of four plants (rice, mustard, cauliflower and onion). We have also studied the possible effects induced by exposure of plant seedlings to MC on the three antioxidant enzymes viz., SOD, POD and CAT.

Materials and Methods

Test Organisms

14 cyanobacterial strains belonging to *Anabaena*, *Nostoc* and *Scytonema* were isolated from rice-fields, bare rocks, wall and polluted pond, whereas *Microcystis* was isolated from thick natural blooms from two local eutrophicated ponds (Durgakund and Laxmikund). *Oscillatoria* sp. growing in winter season in one of the eutrophicated ponds (Laatbhairav) was also isolated. All these isolates were routinely grown as pure, unialgal cultures under laboratory conditions. Identification of all cyanobacterial genera was done on the basis of morphological observations and available taxonomic keys (Desikachary 1959).
Seeds of monocot rice (*Oryza sativa* L.) and onion (*Allium cepa* L.) plants and dicot mustard (*Brassica juncea* L.) and cauliflower (*Brassica oleracea* L.) plants were obtained from Indian Vegetable Research Institute, Adalpura, Varanasi.

Culture Methods and Maintenance

Preliminary test for the presence of bioactive substances was done by using crude aqueous extract of cyanobacterial cells. Cyanobacterial cultures harvested from late exponential growth phase/or natural blooms were used for the preparation of crude aqueous extract. Extract was prepared in sterilized DDW by sonicating cells in a Branson Sonifier-450 (Branson Ultrasonics Corp., Danburry, CT, USA) for 5 min at 8 output and 60 % duty cycle. Sonicated suspension was stirred for 12 h at room temperature. After centrifugation at 10,000 x g for 20 min the resulting pellet was re-extracted 2-3 times. The supernatants were mixed, evaporated to dryness in vacuum and weighed. Known amount of the dried material was dissolved in sterilized DDW to get desired concentration of the extract and filtered through 0.45 μm pore size milipore filter (Millipore Intertech Inc., Bedford, MA, USA) prior to use. Two different concentrations of the crude extract were used for testing bioactivity.

Results

Effect of MC on Seed Germination

Seed germination in all plants began after 24 h, as indicated by opening of the seed coat. Our results show significant inhibitory effect of MC on germination percentage of mustard, cauliflower and onion seeds (Fig. 1). Percent seed germination was not affected much at lower concentrations (0.5 and 1.0 µg/ml) of MC, however higher concentrations (2.0 and 2.5 µg/ml) caused significant decrease in germination percentage of mustard, cauliflower and onion seeds. Percent seed germination in rice was not affected much even upto 2.5 µg/ml concentration of MC and showed only 8 % inhibition (values of percent seed germination were 87 in control seeds and 80 in seedlings grown in 2.5 µg/ml MC supplemented medium) of seed germination, whereas in mustard, cauliflower and onion germination percentage decreased to 57, 52 and 43 respectively at 2.5 µg/ml MC concentration. This corresponded to approximately 37, 45 and 54 % inhibition respectively as compared to the control seeds. It is clearly evident that MC strongly inhibits the germination of mustard, cauliflower and onion seeds of which onion seems to be the most sensitive of the four plants tested.

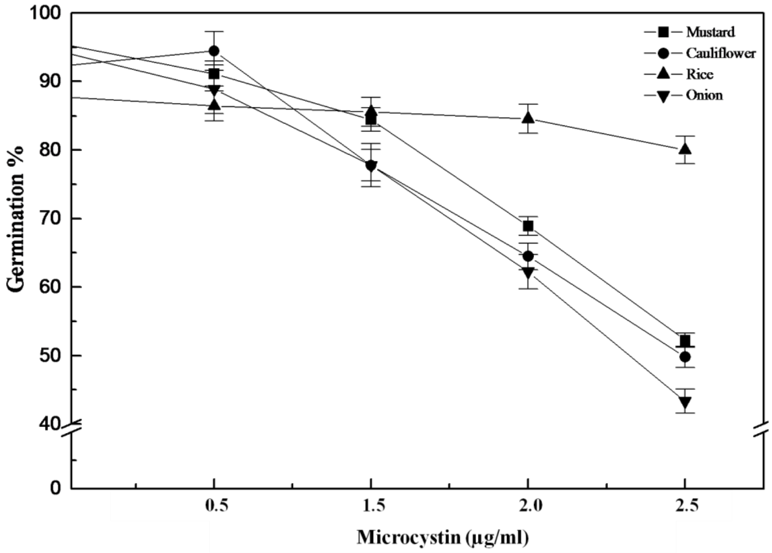

Fig. 1 Effects of MC on the seed germination of rice, cauliflower, mustard and onion.

Effect of MC on Growth and Development of Seedlings

Exposure to MC also resulted in significant inhibition of the growth of seedlings (assessed in terms of shoot and root length) of all four plants tested as compared to control samples. Seedlings exposed to higher concentrations of MC (2.0 and 2.5 µg/ml) exhibited shorter shoots with a yellowish appearance indicating reduced chlorophyll content and most of the MC exposed seedlings had reduced roots. Necrosis in root tips with chlorotic and/or necrotic cotyledons was also observed in mustard and cauliflower. Supplementation of different concentrations of MC in the MS medium significantly inhibited the growth of seedlings in all plants after 5 days. At a concentration of 0.5 µg/ml MC there was no significant difference in shoot length of rice and onion seedlings but higher concentrations of MC (2.0 and 2.5 µg/ml) resulted in a significant decrease in shoot length in all four plants. MC concentration of 2.5 µg/ml caused 53, 46 and 32 % decrease in shoot length of onion, cauliflower (and mustard) and rice respectively (Fig. 2 a). Increasing concentration of MC also produced significant inhibition of root development in seedlings of all plants. Similar to shoot length, inhibition in root length was also not significant at 0.5 µg/ml MC concentration. Upon increasing the concentration to 2.5 µg/ml statistically

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significant reduction in root length occurred in seedlings of all four plants (Fig. 2 b). Reduction in root length was more evident in onion seedlings which showed about 67 % reduction in root length. Rice and cauliflower (mustard) seedlings exhibited 47 and 36 % reduction in root length respectively. Yield, determined by fresh biomass, also decreased significantly in a dose dependent manner (Fig. 2 c). Seedlings exposed to 0.5 µg/ml MC did not show significant inhibitory effect on fresh weight of all four plant seedlings tested. However statistically significant inhibition in fresh weight was observed at higher concentrations. Comparison of the mean fresh weight of rice, mustard and cauliflower seedlings to that of the control indicated 32-40 % decrease in fresh weight at 2.5 µg/ml of MCs concentration. Maximum reduction in fresh weight (66 %) at this concentration was observed in onion.

A profound inhibitory effect of MC was also observed on total chlorophyll content of all the four plant seedlings (Fig. 2 d). At lower concentration (0.5 µg/ml) of MC there was not much decrease in chlorophyll content as compared to control in all seedlings. However chlorophyll content decreased significantly at higher concentration of MC in seedlings of all plants. Mustard and cauliflower shoots showed brown patches and in onion browning of shoot tip occurred at the higher concentration of MC (2.0 and 2.5 µg/ml). Minimum decrease in chlorophyll content occurred in rice which showed only 28 % decrease in chlorophyll content as compared to the control in the presence of 2.5 µg/ml MC concentration. Mustard, cauliflower and onion had 72, 68 and 60 % decrease respectively at the same MC concentration.

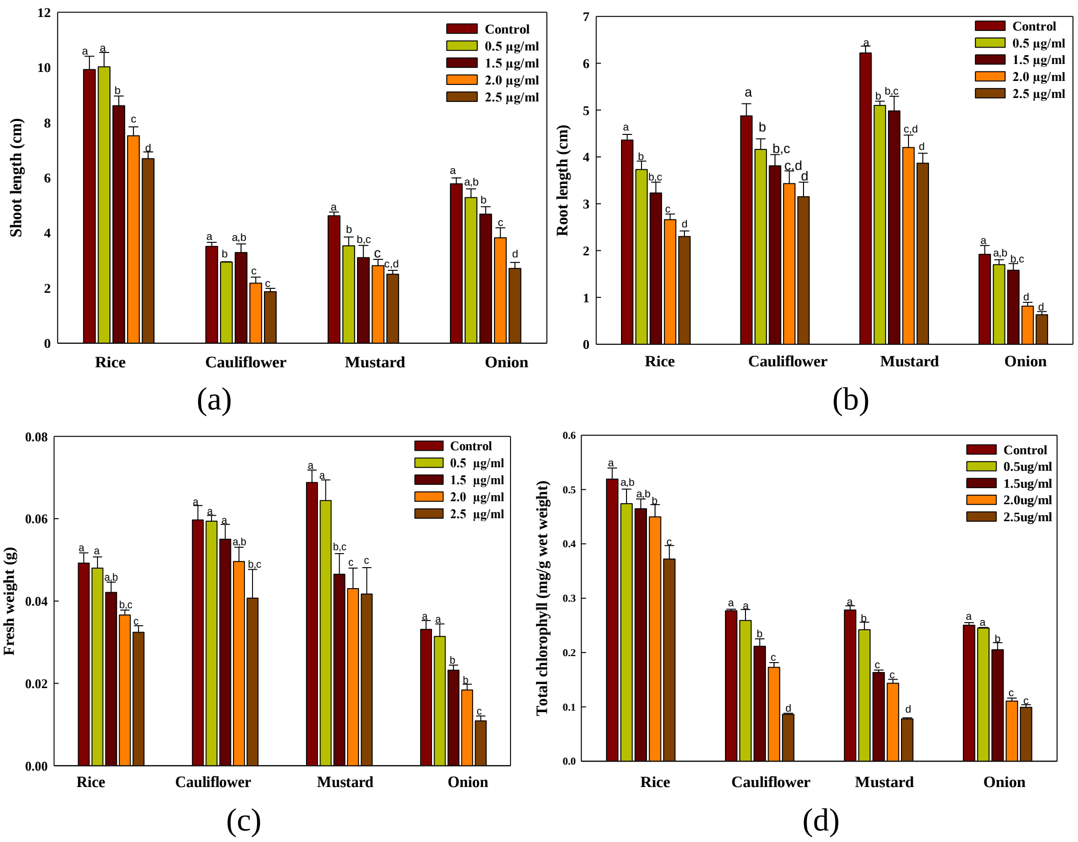


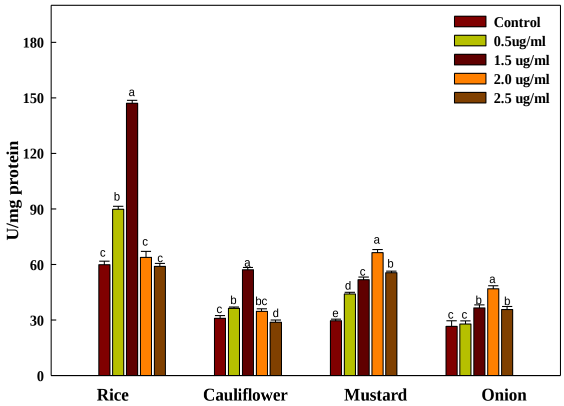
Fig. 2 Effects of MC on the (a) shoot length, (b) root length, (c) fresh weight and (d) chlorophyll content in seedlings of rice, cauliflower, mustard and onion. Observations were made after 5 days of incubation of germinated seeds. Values represent means of three parallel replicates of 30 seedlings. The data are presented as mean value ± standard error. Different letters indicate significant differences between mean values (Duncan's multiple range test P≤0.05).

Effects of MC on the Antioxidant Enzymes of Seedlings

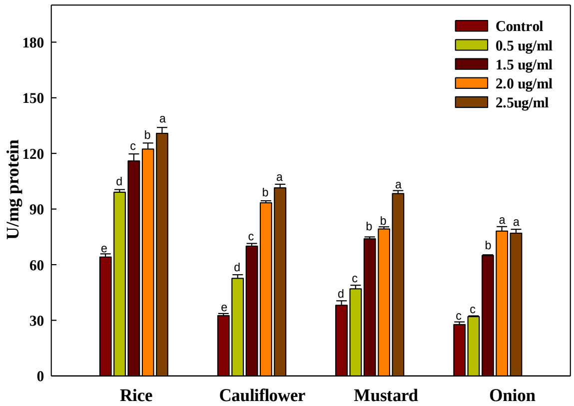
Fig. 3 shows the effect of MC on the activity of SOD, POD and CAT in plant seedlings grown in MS medium. The enzyme activity were measured after 5 days of incubation. It is evident that MC acts as a stress and a concentration dependent significant increase in activity of SOD, POD and CAT was observed in the presence of different concentrations of MC in seedlings of all plants tested. It is pertinent to mention that maximum basal activity of SOD (59.6 U/mg protein) was observed in rice. Maximum increase in SOD activity (upto 2.5 fold) was observed in rice seedlings grown in presence of 1.5 µg/ml MC (Fig. 3 a). Further increase in MC concentration led to decrease in SOD activity, almost reaching to the value found in control seedlings. More or less similar trend in the activity of SOD was also observed in seedlings of other plants (Fig. 3 a). A 1.9 fold increase in the SOD activity was observed in cauliflower at 1.5 µg/ml MC supplementation. However in mustard and onion an increasing trend of SOD activity was observed up to 2.0 µg/ml MC concentration. A 2.3 and 1.8 fold increase in SOD activity in mustard and onion was found respectively at 2.0 µg/ml MC concentration. Further increase in MC concentration up to 2.5 µg/ml led to a decrease in SOD activity. Similarly supplementation of different concentrations of the MC in the medium led to elevated POD activity in seedlings of all plants (Fig. 3 b). Rice showed maximum basal activity of POD (64 U/mg protein). In contrast to SOD where the maximum increase in activity was observed only up to 1.5-2.0 µg/ml of MC concentration in different plants, POD activity showed an increasing trend up to 2.5 µg/ml concentration of MC (Fig. 3 b). At this concentration a 3.12, 2.8, 2.6 and 2.0 fold increase in POD was observed in mustard, onion, cauliflower and rice seedlings respectively. Increase in the activity of CAT in the presence of MC was similar to that of POD activity, where the maximum increase in activity was observed in the presence of the 2.5 µg/ml MC. All seedlings under control condition showed more or less similar basal CAT activity (54 to 63 µmole H2O2/min/mg protein) which increased to 2.2 fold in onion and to 1.8 fold in rice, mustard and cauliflower seedlings at 2.5 µg/ml of MC concentration (Fig. 3 c).

Discussion

In the present study we have reported pronounced decrease in root and shoot development and fresh weight in the presence of MC (2.5 µg/ml) in rice, cauliflower, mustard and onion seedlings. We also observed a drastic inhibition in percent germination of seeds in the presence of 2.0-2.5 µg/ml MC in mustard, cauliflower and onion. However, there was no significant effect of this concentration of MC on seed germination in rice. One distinct feature in MC exposed rice seedlings was the inhibition of primary root elongation at a concentration of 2.5 µg/ml, MCs almost blocked the elongation of primary roots but could not inhibit the growth of lateral roots, so exposed rice seeds still exhibited higher germination percentage. Rice also showed minimum decrease in chlorophyll content in the presence of MC. From our results it appears that the extent of MC induced inhibition is plant specific and plants may show varying degree of susceptibility towards MC. In the past effects of cyanobacterial toxins on terrestrial plants such as inhibition of germination and growth, decrease in chlorophyll content, induction of plant browning and stem malformation, disturbance of signal pathway, inhibition of protein phosphatases and elevation of antioxidant enzyme activities such as SOD, ascorbate peroxidase and glutathione s-transferase have been observed in terrestrial plants (Mackintosh et al. 1990; Kós et al. 1995; Pflugmacher et al. 2006; Sanevas et al. 2006, 2007; Saqrane and Oudra 2009).



(a)



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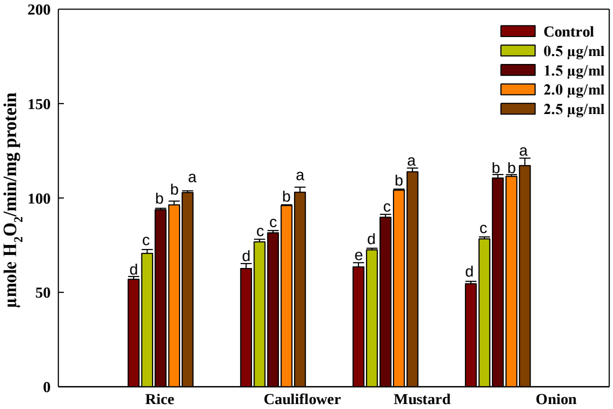

(c)

Fig. 3 Effects of MC on the activity of (a) SOD, (b) POD and (c) CAT of rice, cauliflower, mustard and onion seedlings. Observations were made after 5 days of incubation of germinated seedlings. The data are presented as mean value ± standard error. Different letters indicate significant differences between mean values (Duncan's multiple range test P≤0.05).

The growth inhibitory effect of MCs and crude extract of toxic cyanobacteria was first observed in white mustard (*S. alba*) seedlings (Kós et al. 1995). Kurki-Helasmo and Meriluoto (1998) have reported a dose dependent effect of MC on growth of mustard seedlings. They reported the inhibitory effect of toxin at a concentration of 0.8 µg/ml MC and found that higher concentration of toxin (20-40 µg/ml) almost completely block the formation of root hairs and roots. Sanevas et al. (2006) have shown differential effects of hepatotoxic *Hapalosiphon* sp. extract on root and shoot development in several plants and found prominent cessation of root elongation. They also reported that growth suppression of seedlings was species selective. Several workers (Gehringer et al. 2003; Hamvas et al. 2003; Chen et al. 2004) have reported the sensitivity of plant seedlings (rape and rice) toward extracts of a toxic strain of *Microcystis aeruginosa* revealing growth inhibition with diminished root length, fresh weight plant length and lateral root formation. The reason for inhibition of seed germination and growth of seedlings in the presence of MC might be due to the inhibition of protein phosphatases, which are the key regulatory enzymes involved in many processes of cell division cycles. It has been reported that MCs acts by inhibiting the protein phosphatases and one of the interaction involves covalent bonding of MC to protein phosphatases especially PP1 and 2A (Herfindal and Selhem 2006). This may be correlated to the fact that growth and development in plants depends on regulated cell events and protein phosphorylation and dephosphorylation play essential roles in signal transduction pathways affecting almost all regulatory pathways of plant metabolism *in vivo* (Smith and Walker 1996; Saqrane and Oudra 2011).

Although in the present study we have not studied the uptake and accumulation of MC in plant tissues however uptake of MCs by roots of various terrestrial plants seedling followed by their translocation and accumulation in greater quantities in plants have been shown by several workers indicating that different plant species may accumulate MCs at different rates and plant parts depending upon plant species and MCs variant (Chen et al. 2004; Crush et al. 2008; Saqrane et al. 2009; Liang and Liu 2020). Accumulation of sufficient concentration of MCs in terrestrial plants has been known to occur and various studies have reported the uptake of MC-LR by submerged and emerged aquatic plants (Pflugmacher 2002; Yin et al. 2005b). In terrestrial plants, crop irrigation by water containing cyanobacteria can lead to an exposure of aerial parts of plants to cyanobacteria and their toxins (Abe et al. 1996). By using commercialized ELISA kit presence of MC have been detected in the tissues of exposed plants suggesting that the uptake and accumulation of these toxins in plants may occur and also affect human health (McElhiney et al. 2001). These workers showed the phytotoxic effects of MC on the growth and development of potato (*Solanum tuberosum*) and runner beans (*Phaseolus vulgaris*). Chances of accumulation of high MC concentration in soil are high during irrigation with MC contaminated water as MCs are considered quite resistant to degradation (Harada 1996). Because of their cyclic structure they are chemically very stable (Lahti et al. 1997) and remain in water for a long period (Watanabe et al. 1992).

It has been shown previously that reactive oxygen species (ROS) such as singlet oxygen ($_1O^2$), superoxide anion ($O_2.^-$), hydrogen peroxide (H_2O_2) and hydroxyl (OH**.**) and hydroperoxy radicals (HO_2**.**) are generated during plant metabolism, especially in the plants exposed to environmental stresses and they need to be scavenged for maintenance of normal growth. A large number of evidences have proved that environmental stresses alter the amounts and the activities of enzymes involved in scavenging oxygen radicals (Mittler 2002). Among these enzymes, SOD, POD and CAT are the important enzymes active in elimination of ROS. MC contaminated water could be one of abiotic stresses for plants. In view of this, we have investigated the activities of few antioxidative enzymes viz., SOD, POD and CAT in seedlings grown in MC supplemented medium. Our aim was to test whether a general oxidative stress is induced by MCs. The results of this study demonstrated that the activities of SOD, POD and CAT in rice, cauliflower, onion and mustard were affected in a concentration dependent manner. Our findings suggest that SOD, POD and CAT may take part in alleviating the MC induced stress in these plants. As compared to other plants tested, rice posses the maximum basal activity of SOD and showed maximum increase in SOD activity (2.5 fold) at 1.5 µg/ml concentration of MC. Increasing of MC concentration upto 2.5 µg/ml decreased the SOD activity but increased the activity of POD and CAT.

Our results are in agreement with results of Chen et al. (2004), who demonstrated that lower concentrations of MCs (0.024-0.12 µg/ml) significantly increased SOD and POD activity in rice (*Oryza sativa* L.) (Cao et al 2018) and rape (*Brassica napus* L.) seedlings, whereas higher concentrations (0.6-3.0 µg/ml) only slightly increased SOD activity but induced the activity of POD in rape seedlings. Several workers have shown that exposure to 5 µg/l MC-LR for 2 h to the aquatic macrophyte *Ceratophyllum demersum*, or 10 days to alfalfa (*Medicago sativa*), significantly increased SOD activity (Pflugmacher 2004; Pflugmacher et al. 2006). Furthermore, exposure to MC-LR concentrations as low as 0.5 µg/l increased SOD activity in spinach (*Spinacia oleracea*) variants (Pflugmacher et al. 2007).

Pflugmacher et al. (2007) also studied the effect of different cyanobacterial toxins on induction of oxidative stress and seed germination in alfalfa (*Medicago sativa*) and reported marked increase in SOD, POD and CAT enzyme activities at 5.0 µg/L toxin concentration after 7 d exposure. These results suggest that SOD, POD and CAT are sensitive indicator of oxidative stress produced by MCs in various plants (Yin et al. 2005a). It has been proposed that in addition to their toxicity component MCs are capable of causing oxidative stress and SOD, POD and CAT may take part in alleviating the stress induced by exposure to MC. Most of the investigations into the effects of MCs on plants suggest that exposure to MCs via irrigation with MC contaminated water presents a threat to the quality and yield of crop plants in the environment and highlight the need to examine the level of toxin which may be detrimental to crops. Although the MC concentration used in the present study may not represent the toxin level present in natural water however MC concentration may increase in soil by repeated use of MC contaminated water during irrigation. Moreover as MC are considered quite resistant to degradation (Harada 1996) accumulation of high MC concentration may occur in soil. Under some circumstances some of the water bodies may show a high concentration of MC (1.3-1.8 µg/ml) in water (Jones and Orr 1994) which is considerably higher than the values (1 µg/L) mentioned as unsafe for drinking purpose as per WHO guidelines.

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