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Impact of cyanobacterial toxins (microcystins) on growth and root development of *in vitro Vicia faba* cultures

Majida Lahrouni[1-2], Khalid Oufdou[1], Fatima El Khalloufi[1-3], Eloisa Pajuelo[2], and Brahim Oudra[1]

[1]Laboratory of Biology and Biotechnology of Microorganisms, Environmental Microbiology and Toxicology Unit, Faculty of Sciences Semlalia, Cadi Ayyad University, PO Box 2390, Marrakech, Morocco

[2]Departamento de Microbiología y Parasitología, Facultad de Farmacia, Universidad de Sevilla, Spain

[3]Univ. Hassan 1, Faculté polydisciplinaire de Khouribga, BP. 145, 25000 Khouribga, Morocco

ABSTRACT: The occurrence of toxic freshwater blooms of cyanobacteria has been frequently reported during the last 15 years in Lalla Takerkoust lake (35 km southwestern of Marrakech city) In this study, it has been confirmed that the collected cyanobacteria bloom could produce different variants of microcystins at high concentration of 11.5 mg equiv. MC-LR g^{-1}DW of the cyanobacteria cells. In order to study the effect of microcystins on faba bean seedling cultured *in vitro*, the crude aqueous extract of the toxic bloom was prepared and sterilized by filtration, and then it was supplemented to BNM medium at different concentrations. After 10 days of *in vitro* seedlings growing in BNM medium, plants fresh and dry weights were determined, plants shoot and root length was measured and then the roots were subjected to histological microscope observation of root hair, root tip and root cortical cells. The results revealed that microcystins exposure induced a decreasing of seedling growth and biomass accumulation in a concentration dependent manner. In addition, seedling young roots exhibited a brownish aspect, necrosis and tissue lysis. At a microcystin's concentration of 40 µg/mL equivalent MC-LR, the root elongation and root hair formation, were blocked almost completely. At concentrations 10-80 µg/mL equivalent MC-LR the root tips exhibited necrosis and tissue lysis. Concentration of 10 µg/mL equivalent MC-LR induced a reduction of cortical cell size. These results indicated that microcystins contamination introduced via irrigation water from Lalla Takerkoust lake, could have a negative effect on crop yield in Marrakech El Haouz region.

KEYWORDS: Cyanobactria, Faba bean, microcystins, root hair, root tip, cortical root cells, irrigation.

1 INTRODUCTION

The occurrence of cyanobacterial blooms in surface water mainly used for spray or spreading irrigation has received increasing attention over the last decade due to their ecotoxicological potential effects. Bloom-forming-cyanobacteria often produce highly toxic microcystins (MCs), including microcystin-LR (MC-LR), that is one of the most studied cyanotoxin [1], [2]. Exposure to MCs via irrigation water has a negative impact on the quality of crop yield. For instance, it has been reported that MCs have a negative effect on plant biomass accumulation, plant growth, photosynthesis, nutrient absorption and seeds germination [3], [4], [5]. It has been reported also that MCs induce oxidative stress in seedlings of several agricultural plants [6], [7], [8]. In addition, several authors have reported that MC-LR could be absorbed by roots and be translocated from roots to shoots and the fruit [9], [10], [11], [12], [13], which can constitutes an important public health problem [14].

Faba bean is considered as the major leguminous crop in Marrakech-El Haouz region that could be exposed to MCs via irrigation water from Lalla Takerkoust lake. Therefore, this plant was chosen as a model to study the effect of MCs on plant growth and development. In our previous studies we have demonstrated that MCs have a negative effect on nodulation process and biological nitrogen fixation of faba bean seedling in symbiosis with different rhizobia strains [15], [8]. We also

reported that root part is more sensitive to MCs that did shoot part [8], this can be explained by the fact that the roots are the organ where nodulation take place, and it is the first organ to deal with this MCs stress.
This study was performed in order to study the effect of the water crude extract of cyanobacteria containing MCs on the growth and development of faba bean seedlings cultivated *in vitro* (by this manner, we simulate a real environmental conditions allowing the contamination of plant crop by cyanobacterial toxins) . Particular attention was given to the effect of different concentrations of MCs on the growth of primary roots, root hair formation, tip formation and roots histological changes.

2 MATERIALS AND METHODS

2.1 CHARACTERIZATION AND QUANTIFICATION OF MICROCYSTINS FROM CYANOBACTERIAL BLOOM

The cyanobacterial bloom material (*Microcystis aeruginosa*) was collected in October 2010 from "Lalla Takerkoust" reservoir (Marrakech, Morocco), and was freeze-dried. MCs concentration was determined using the protein phosphatase type 2A inhibition assay according to [16]. Qualification of MCs was determined using High Performance Liquid Chromatography (HPLC) system as described in [17]. The toxin equivalent concentration in *M. aeruginosa* bloom was expressed as mg MC-LR equivalent (equiv.) $.g^{-1}$DW.

2.2 PREPARATION OF MICROCYSTINS CRUDE EXTRACT

Freeze-dried cyanobacteria were suspended in distillated water, grinded and then centrifuged at 20 000g for 30 min. The supernatant containing MCs was sterilized by filtration using sterile syringe filters (0.45 µm pore diameter) and kept at -20°C until further use.

2.3 BIOLOGICAL MATERIAL AND PLANT GROWTH CONDITIONS

Commercial faba bean seeds (*Vicia faba* L. var. Alfia 5) were surface sterilized using sodium hypochlorite (6°) during 15 min and then rinsed several times with sterile distillated water. The seeds were then pre-germinated at +26°C in sterile Petri dishes (9 cm diameter) containing 20 mL agar agar 5%. The pre-germinated seed were then placed in sterile tubes (15 cm de length and 18 mm de diameter) containing 10 mL of Buffered Nodulation Medium (BNM) [18], pH 6.5, that was solidified with 1.2% plant agar, and supplemented with various concentrations of MCs 0 (control), 2.5, 5, 10, 20, 40, 80 µg/mL equiv. MC-LR. Twelve tubes (replicates) for each MC concentration were prepared. The tubes were incubated at +22°C with a 16/8 h day/night photoperiod.

2.4 PLANT HARVEST AND ANALYSIS

The experiment lasted 10 days. After this time, plants were detached from BNM medium agar, washed and blotted dry on filter paper. Seedlings from each treatment were separated into two groups (6 plants per group). For one group, the whole plants fresh weight and shoot and root lengths were determined immediately. And then, the whole plants dry weight was recorded after 72 h at 70 °C. For the other group, the primary roots were used for microscope observation of root hair formation, root cap formation and for histological investigations. A Motic DMBA-310 Compound Digital Microscope equipped with a digital LED camera was used.

2.5 STATISTICAL ANALYSIS

The experimental design was a randomized complete block. Data regarding plants fresh and dry weight and shoot and root length, were means of six replicates per treatment. Data were analyzed by variance analysis (ANOVA), and the mean separation was achieved by LSD test by the COSTAT software. All numeric differences in the data were considered significantly different at the probability level of $P \leq 0.05$.

3 RESULTS

3.1 CYANOBACTERIAL BLOOM ANALYSIS

The PP2A analysis of the cyanobacterial bloom (*M. aeruginosa*) collected in October 2010 showed a total MCs concentration of 11.5 mg MC-LR equiv. g^{-1} DW [19]. The HPLC chromatogram revealed the presence of one MC variant that was identified as MC-LR using the commercial standard.

3.2 EFFECT OF MCS ON THE GROWTH, BIOMASS ACCUMULATION, ROOT HAIR FORMATION, ROOT CAP FORMATION AND ROOT CORTEX CELLS OF *V. FABA* SEEDLING CULTURED *IN VITRO*

Exposure to MCs significantly inhibited the growth and biomass accumulation of faba bean seedling in a concentration dependent manner (figs. 1, 2 and 3), roots being more affected than shoots. Indeed, for MCs concentrations of 2.5, 5, 10, 20, 40, and 80 µg/mL equiv. MC-LR, the reduction rates of root growth are respectively 2.3, 3.5, 2.3, 2, 1.5 and 1.03 fold higher than those of shoot part. Moreover, at the end of the experiment, no necrosis, no brownish aspect and no visual morphological changes were perceptible in the shoot part for any MCs tested concentrations compared with the controls (figs. 1 and 2). The seedlings which were exposed to different MCs concentrations exhibited shorter shoots than the control but looked normal (fig. 1). While, remarkable visual changes were noticed on root of plants exposed to MCs, After 10 days, the roots of seedling grown in the presence of MCs were turning brown and looked thinner than those of the controls (fig. 1), the seedling at the concentration 10 µg/mL equiv. MC-LR or higher had malformed lateral roots, and as the concentration of toxin increased these effects became more evident (fig. 1). At higher concentrations ($\geq$ 20µg/mL) faba bean seedling had no primary roots. This could be explained by the fact that roots are in contact with MCs so they are the first organ to deal with these toxins.

In this study, we also reported that MCs exposure had a negative effect on root hair formation, root tips formation and caused a reduction of root cortical cells size (fig 4, 5 and 6). Indeed, the negative effect of MCs was apparent from the lowest concentration tested, and it increases with the increasing concentrations of MCs. For example, concentrations ranged from 2.5 to 20 µg/mL equiv. MC-LR decreased the number and the length of root hair, and concentrations of 40µg/mL and 80µg/mL equiv. MC-LR blocked the formation of roots and root hair almost completely (figs. 1 and 4). In addition, at 10 µg/mL the root tip exhibited necrosis, and at the concentration 20 µg/mL equiv. MC-LR or higher the root tip exhibited necrosis tissue, brownish aspect and tissue lysis (fig. 5).

4 DISCUSSION

The exposure of terrestrial plants to MCs via irrigation water taken from a source that has experienced a toxic cyanobacterial bloom containing MCs has far reaching consequence for both economic and health reasons [20], [21]. Growth inhibition of seedling of a variety of terrestrial plants by MC [22], [21], [23], the inhibition of protein phosphatase [24] and photosynthesis in leaves of terrestrial plants [25] have been reported. This study revealed that MCs concentrations of 2.5-80 µg/mL equiv. MC-LR have toxic effects on the growth, biomass accumulation, root hair formation, root tip formation and root cortical cells of *V. faba* seedling cultured *in vitro*. The MCs concentrations that caused adverse effects in the present study are similar to those reported for other terrestrial plants cultured *in vitro*. For example, the growth and development of *B. napus*, *M. pumila* and *O. sativa* seedling were inhibited after exposure to 3 µg/mL MCs during 4 days (*B. napus* and *O. sativa*) and 14 days (*M. pumila*) [7], [10], [23]. Same result was found for *S. tuberosum* and *C. demersum* after their exposure to 5 µg/mL MCs during 16 days and 24 hours respectively [21], [26]. Furthermore, Mathé et al. [27] studied the effect of pure MC-LR (2.5-80 µg/mL) on the growth and histology of *P. australis* cultured *in vitro* for 35 days. The authors reported the inhibition of the growth of shoot and root parts, histological alterations, brownish aspect, necrosis and tissue lysis.

In this study, we reported that 40 µg/mL equiv. MC-LR completely blocked root hairs formation and root tips exhibited necrosis. Similar results were reported by Kurki-Helasmo and Meriluoto [24]. These authors have shown that concentrations of 40 and 20 µg/mL MC-RR almost completely blocked root elongation and root hair formation of mustard seedlings. Similarly, Yin et al. [28] reported that exposure of *V. natans* to 10 µg/mL pure MC-RR for 30 days caused a significant reduction of fresh weight and root elongation, and inhibited root hair formation. Moreover, Chen et al. [23] reported that root tip exhibited necrosis with chlorotic or (and) necrotic cotyledons after exposure of *B. napus* and *O. sativa* to 3µg/mL MCs. In addition to their inhibitory effect on root hair formation in *V. faba* seedling, MCs also caused a reduction of the size of cortical root cells. Indeed, MCs are inhibitors of protein phosphatase and it is affirmed by [29] that the inhibitors of protein phosphatase such as okadaic acid and calyculin-A inhibit root hair formation and alter the shape of root cortical cells of

Arabidopsis. Similar results were found by [30] that showed histological changes of roots of *P. sativum* after exposure to 11.6 µg/mL equiv. MC-LR.

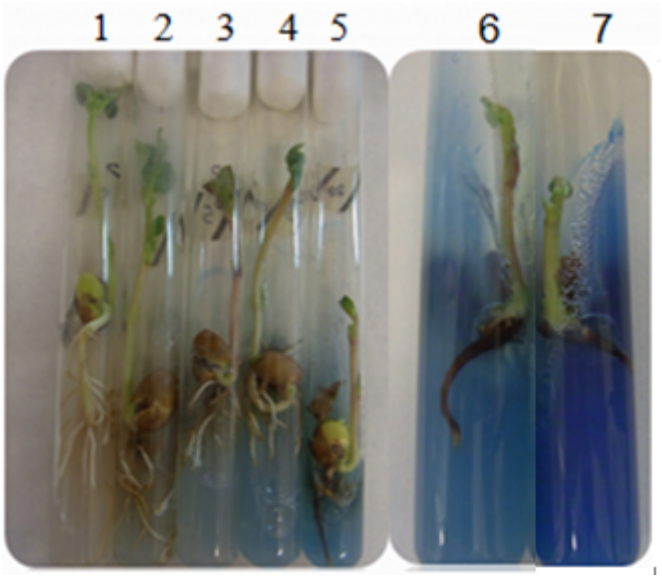

Figure 1: Vicia faba seedling after 10 days exposure to microcystins at different concentrations (from the left to the right) 0 µg/mL (tube 1), 2.5 µg/mL (tube 2), 5 µg/mL (tube 3), 10 µg/mL (tube 4), 20 µg/mL (tube 5), 40 µg/mL (tube 6) et 80 µg/mL (tube 7).

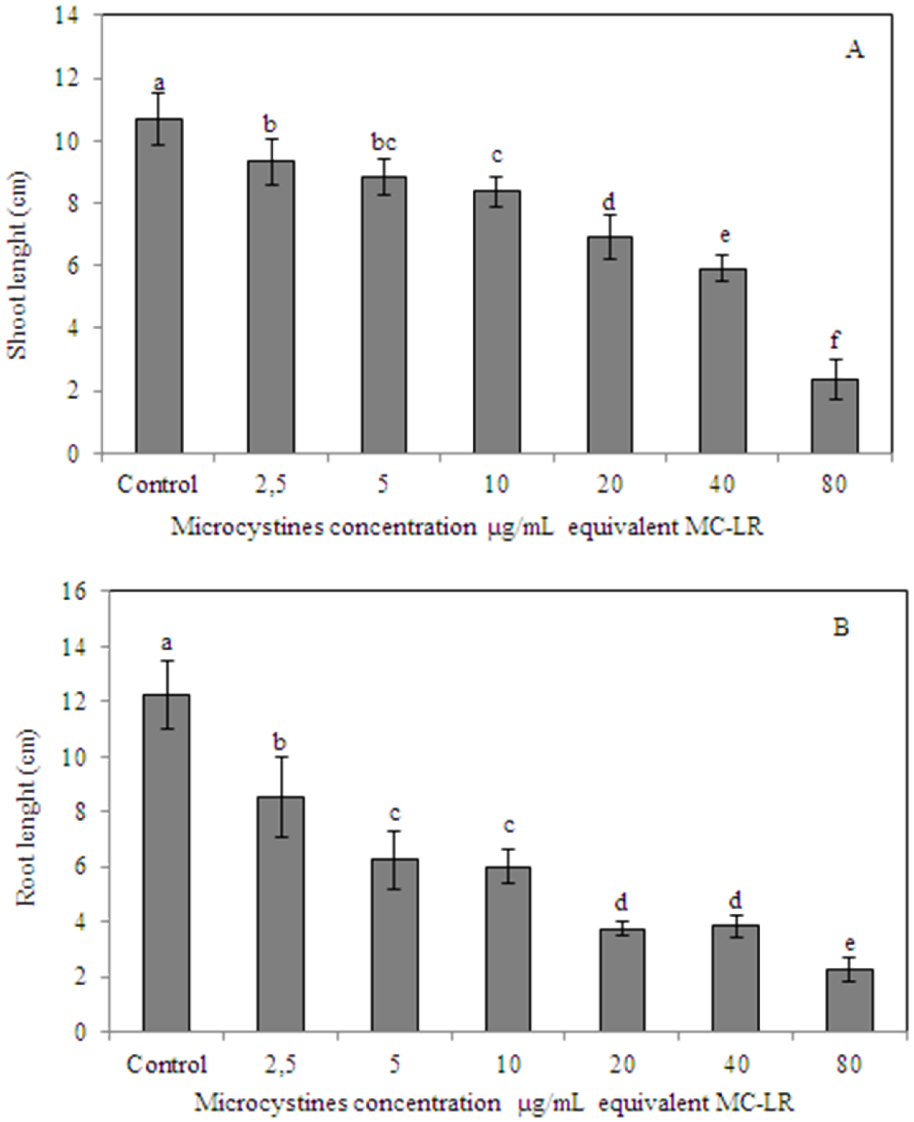

Figure 2: Effect of different concentrations of microcystins on the growth of shoot (A) and root (B) of Vicia faba seedling cultured in vitro for 10 days.

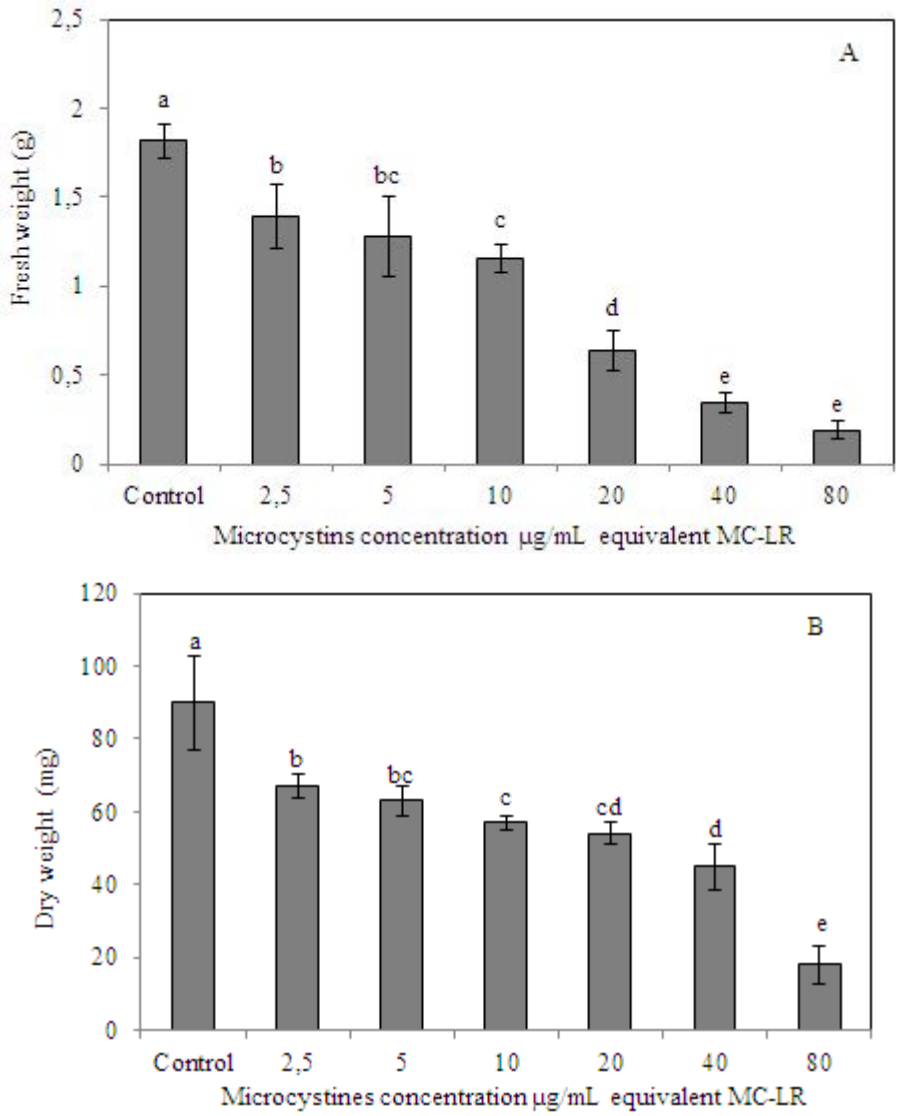

Figure 3: Effect of different concentrations of microcystins on biomass accumulation of Vicia faba seedling cultured in vitro for 10 days, Fresh weight (A) and dry weight (B).

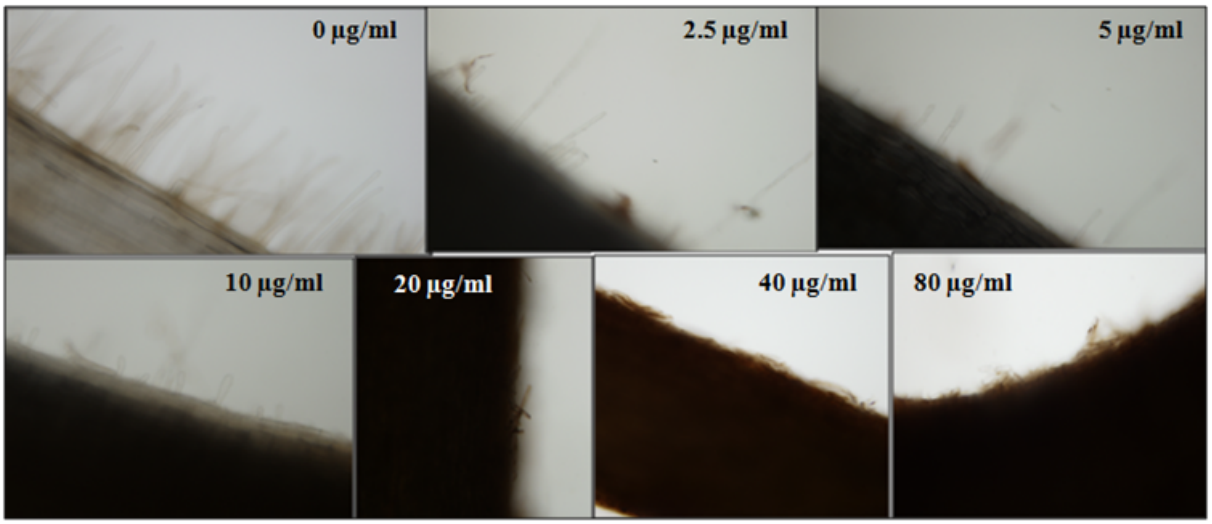

Figure 4: Effect of different concentrations of microcystins on root hair formation of Vicia faba seedling cultured in vitro for 10 days, (G: x400).

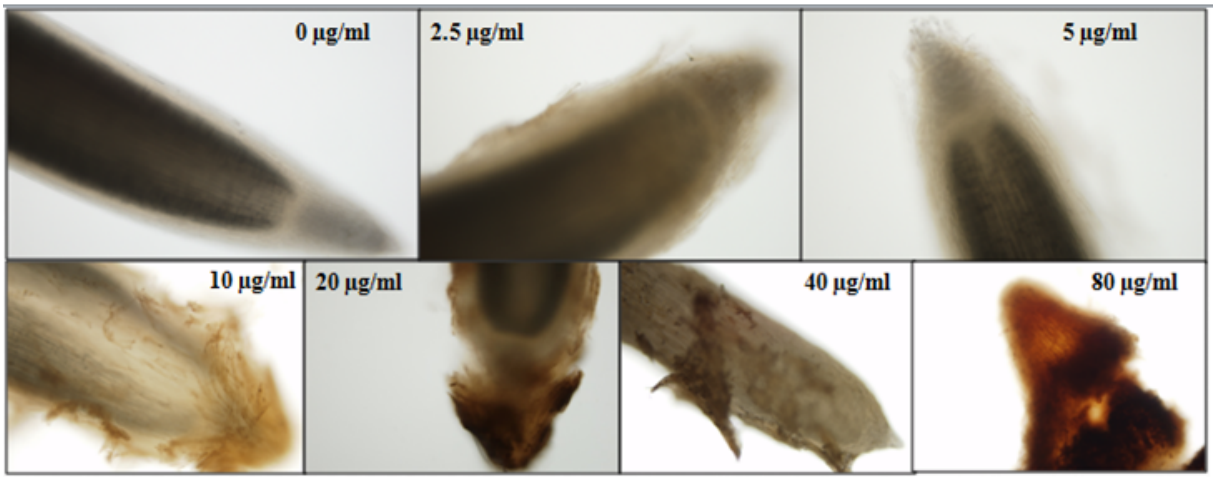

Figure 5: Effect of different concentrations of microcystins on root tip formation of Vicia faba seedling cultured in vitro for 10 days, (G: x400).

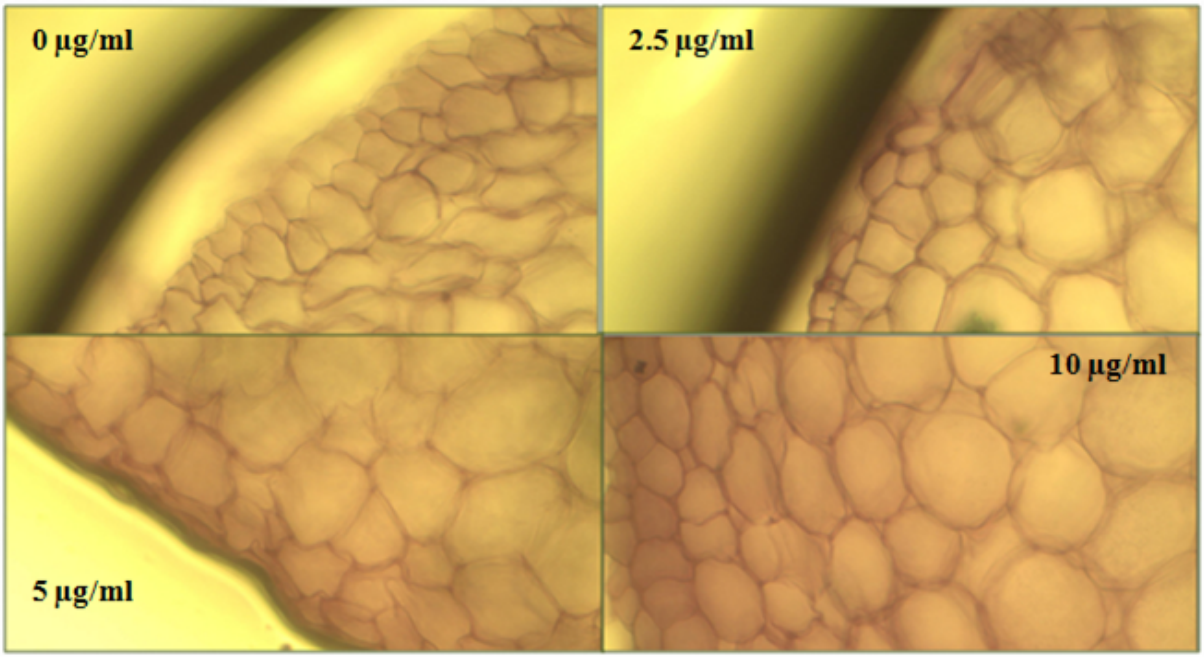

Figure 6: Effect de different concentrations of microcystins on root cortical cellsof Vicia faba seedling culturedin vitrofor 10 days, (G x400).

5 CONCLUSION

According to these results, we can state that exposure to MCs via irrigation route poses a threat to the yield and the quality of the crop by affection plant growth and development. Root part was more sensitive than shoot part, since the growth of roots was more sensitive to MCs than shoots. In addition, no morphological changes, no necrosis and no tissue lysis were observed in shoot part, while root part exhibited necrosis, tissue lysis and brownish aspect. Moreover, MCs at high concentrations blocked root hair formation almost completely and reduced the size of root cortical cells.

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