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Research Article

Phytoremediation of Ethidium Bromide by Tomato and Alfalfa Plants

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Abstract

Phytoremediation is a method of choice for remediates any pollutant from nature. This nascent technology has already undergone successful pilot study and it is time to find more popular and useful plants and bring them to the field to clear the nature. Alfalfa and tomato as a part of traditional culture of many countries could be proper candidates for this purpose. EtBr as a DNA staining dye is one of common tools of molecular biologist. But, the hazardous effects of them (in use or waste) make a new effort to remove safely them after usage. This study examine the alfalfa and tomato`s role in absorbance of EtBr and the subsequence of such accumulation. Seeds of alfalfa and tomato were transplanted and after 23 days in which they grown in appropriate size, we started to inoculate them with several concentration of EtBr. After 10th and 17th day the samples were collected and the analysis significantly demonstrated the diverse effect of EtBr on short and long term on them. In summary, EtBr divided the plants growth to two main phases, before day 10, increasing growth and after day 10, decreasing growth. Increasing roots number and diameter in contrast to decreasing the length of shoots were the special efforts of our research.

INTRODUCTION

The generic term of "phytoremediation" consist of the Greek prefix phyto (means plant), attached to the Latin root remediation (means to correct or remove an evil) [1]. Phytoremediations are promising plants in a contaminated matrix to remove contaminants from a matrix or degradation (detoxification) of the pollutants. Phytoremediation is a mechanical conventional and solar-energy clean-up technology which is an ecologically friendly [2]. For more than 300 years such kind of phytoremediation activity to clean-up the soil pollutants like metals, chemicals, bio-molecules, explosives, oil and pesticides has been recognized and nowadays this technology were used as a non-destructive and cost effective technology that used for removing the hazardous chemicals [3]. Ethidium Bromide (EtBr) is commonly used as a non-radioactive DNA stain (as an intercalatory agent) to identify and visualize nucleic acid bands in electrophoresis. EtBr powder moderately soluble in water and in exposure to UV it will fluorescence a reddish-brown color. EtBr is a potent mutagen and cause genetic damage [4]. Actually, the EtBr is defines as harmful by inhalation, ingestion or skin absorption and in the other hand it causes eye, skin, mucus membrane and upper respiratory tract irritation [5,6]. Uera in 2007 reported the hazardous effect of EtBr on disrupting efficient metabolism, protein synthesis in plants and growth affection on 30 days exposure [6]. Tomato (*Solanum lycopersicum*) is a South America native culture which is growing in temperate climates of world wild [7-9]. Beside tomato, alfalfa (*Medicago sative*) with high salt resistance used as a potential tool to phytoremediate the contaminated soils [10]. It is necessary to develop sustainable and environmental friendly technique in order to remediate the EtBr waste from the soil. With such mentioned advantages there is a bright future for the phytoremediation of contaminated soils [11]. There is no many plant species which have been identified for their traits in the uptake and accumulation of EtBr [6], so in this study we try to find out two tomato and alfalfa plants phytoremediation potential.

MATERIAL AND METHOD

Plant preparation

In this study we use alfalfa (*Medicago sativa*) and tomato (*Solanum lycopersicum*) plant to study the EtBr (Merck, USA) absorption effect on plants. Seeds of both of tomato and alfalfa were obtained from local stores (because of accessibility of them).

Morphological analysis

The plants were inoculated with several concentration of EtBr (4.4, 42, 64, 113, 154 mg/ml). The morphological changes of plants were analyzed after harvesting (10^{th} and 17^{th} days). The length of roots and stems, color, the number of root hairs and special damages on root, stem or even leaves were measured and calculated.

Phytotoxicity assay

The sensivity of two test plant to EtBr were estimated in inoculated soils. Effective concentration that gives 50% reduction (Ec50) was determined by using reduction percentage (%R) calculation according following formula: %R=(1-A/B)×100, where A is the length of shoot or root (treated plants) and B is the length of shoot or root (untreated plants).

Statistical analysis

The several experimental groups of plants were analyzed in day 10 and 17. The morphological changes were observed by eye and measured by ruler. The growth reduction were calculated in contrast to control`s growth rate base (the differences between growth of root from $0^{th}$-$10^{th}$ and $10^{th}$-$17^{th}$ days or stem`s growth per control`s one). All treatments were replicated three times and is expressed as mean ± S.D. Differences between experimental and control groups were determined using the T test. Values of $P < 0.05$ were considered significantly.

RESULTS

EtBr inoclusion of plants and morphological changes

The EtBr toxicity effects on two selected plants at the first glance were not obvious, but the details of analysis indicated the hazardous effects. In each analysis, three out of the ten plants were screened and showed remarkable changes on them. The plants growth and morphology were compared to the control plants which were received just distilled water. Except control in all the experimental alfalfa plants, the roots keep growing until day 10, but significantly after that not only the growth speed decreased but also the roots were shortened. With increasing the EtBr concentrations the growth decline were grown more remarkable. Actually, in the maximum concentration (154 mg/ml) we saw the maximum trendline which indicate the maximum effect. Interestingly, with decreasing the EtBr concentration also the trendline of decline of growth decreases (Figure 1A). Tomato roots showed the same manner and similarly after day 10 and with continuing the inoclusion by EtBr started to reduce the growth. Indeed, the control and the plants which inoculate with the 4.4 mg/ml EtBr stay in the same line apposite the other`s. It seems that the 4.4 mg/ml concentration in tomato had a little and even approximately no significant effect on root growth in our experiment time (Figure 1B). in the other view, although the whole pattern for the growth of the tomato and alfalfa`s shoot almost were similar to their root`s growth, alfalfa`s shoot just affected from two highest concentrations (154 and 113 mg/ml) and the other concentration`s effect remarkably reduce with their reduction. In the overview, directly the concentration increasing trendline were sympathy with the effectiveness of them on plant`s shoots (Figure 2A). Tomato`s shoot exactly similar to alfalfa`s root showed growth reduction in contrast to control. But, you can see the growth trendline from 0-10^{th} day is sharp and from day 10-17^{th} is a little mild (Figure 2B). In all the samples with growth reduction we observe the morphological changes too. They were look like wizened plants and it seems the resultant reduction in their lengths because of their plasmolysis not losing the cells. Morphological analysis also indicated that despite the root growth reduction, the root hairs number were increase. In the test plants (tomato and alfalfa) which were inoculated by higher EtBr concentrations we found the most number of root hair and thicker than normal is crowded. Overall, the growth of the roots and shoots of tomato in contrast to alfalfa`s growth shows more notable decrease. Plants which were inocluated by EtBr

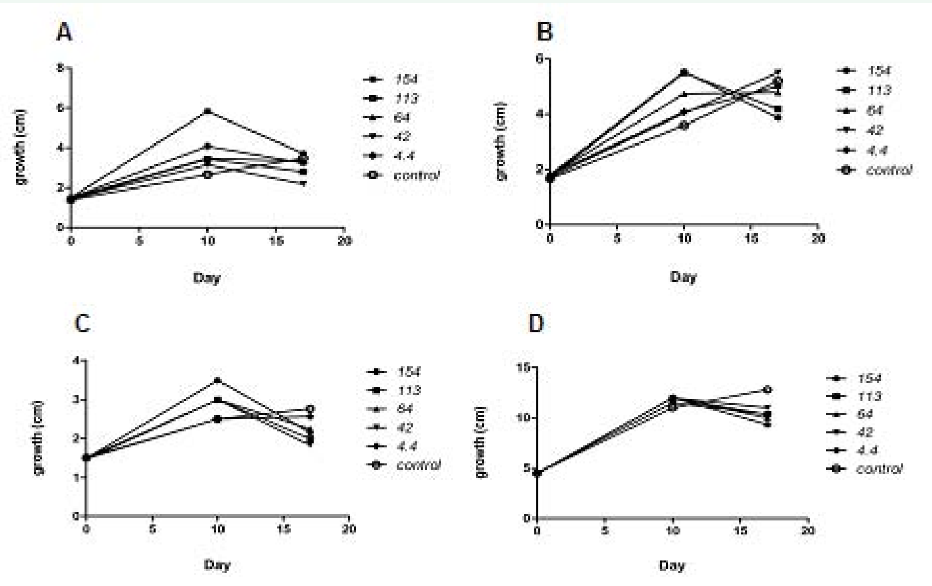

Figure 1 Alfalfa and tomato plants growth during 17 days.
Graph A and B belongs to alfalfa and two other C and D show the tomato`s growth length per day.

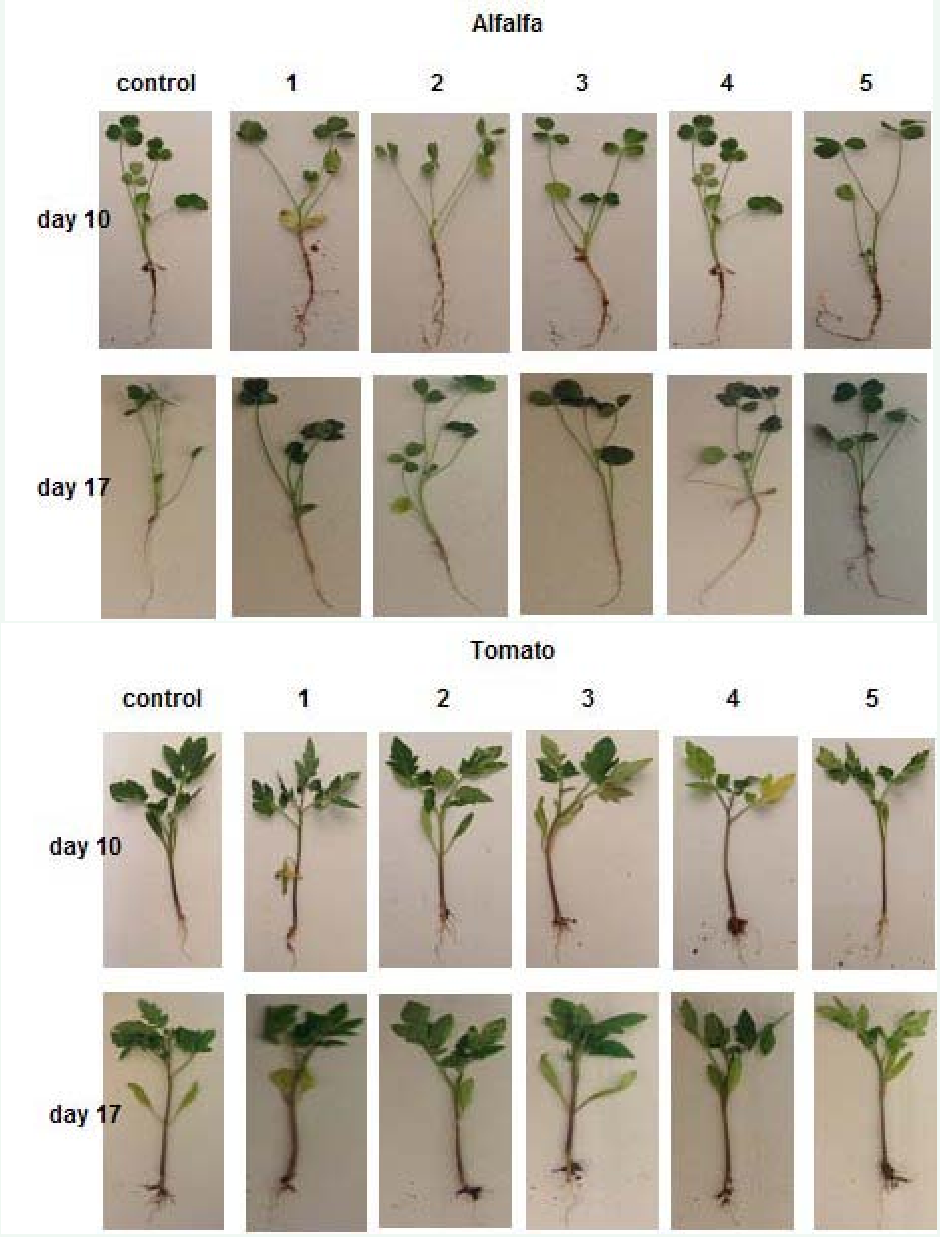

Figure 2 Morphological changes of test plants during treatment.
The serial changes of alfalfa and tomato morphology in day 10 and 17 samples in control and 5 EtBr concentrations treated plants (1: 4.4, 2: 42, 3: 64, 4: 113, 5: 154 mg/ml).

in all concentrations after day 10 were started to withering and yellowing. The color changes from the lowest concentration (4.4 mg/ml) to highest (154 mg/ml) were coming more remarkable (Figure 2). Actually, in the plants both tomato and alfalfa with the 154 mg/ml EtBr reception, the leafs were yellow and the root`s thickness were more notable. Considerably, the tomatoes with 42 mg/ml concentration in both day 10 and 17 samples showed a pied leafs which was not seen in other test or control plants. In general, we observed wanes from dark green in control leafs to lighter green even yellow in the test in a serial dilution of EtBr.

Phytotoxicity results

The phytotoxicity assay in one hand was for assessing the cytotoxicity of EtBr on plant and in the other hand was for finding the specific relation between shoot and root growth changes inside the plants, then compare their results together. As we mentioned before, the decreasing in growth were analyzed by the rate of growth differences of test day 10 and day 17 sample growth to control`s one. The positive and negative numbers show the increasing and decreasing trend of growth from day $10^{th}$ to $17^{th}$. In general, as it is obvious in the first ten days not only we do not have growth retardation but also the test group`s growth (both shoot and root (Figure 3)) were increasingly rise. In contrast, in the last 7 days such affinity was decreased and the graph remarkably falling down. The root of alfalfa and tomato both in a similar manner significantly, even in low 4.4

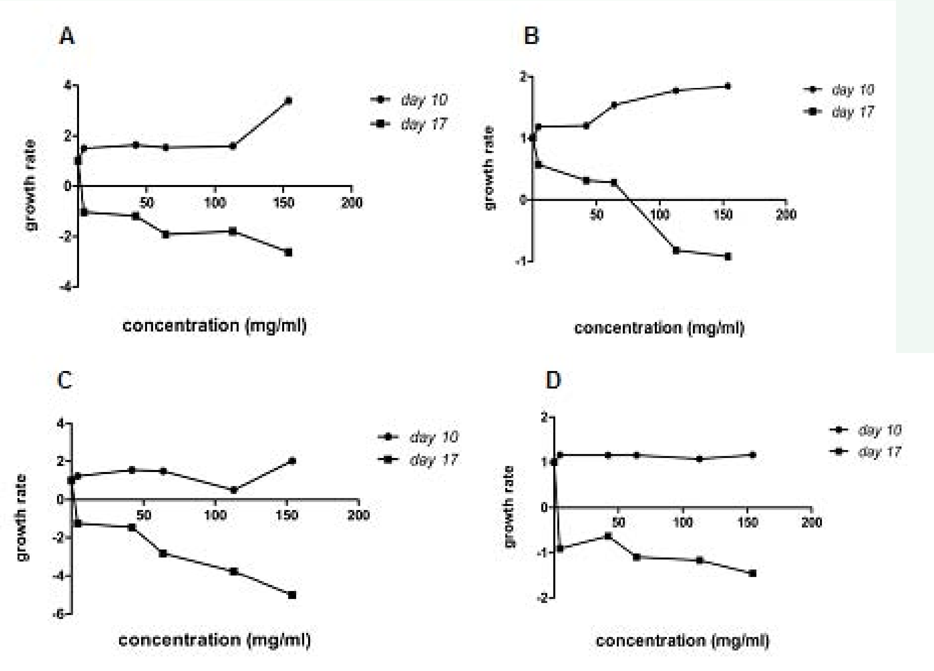

Figure 3 Growth rate of shoot and roots of the test plant against control one.
The ratio of growth length differences from day 10 to 17 were calculated and the results (positive: increase and negative: decrease numbers) of test plants to control. Alfalfa results: graph A and B. Tomato results: graph C and D.

mg/ml concentration of EtBr, showed huge decline in growth. Although, the shoot of alfalfa in contrast to tomato`s root had less decrease in growth, we observed such descending graph (Figure 3B,3C). Furthermore, we divided the growth phase of the roots and shoots (in both plants) to two increasing (before day 10) and decreasing (after day 10) phase. Additionally, as you can see in (Figure 3), the effect of higher concentrations was more significant than the lower`s. In the other assay, we calculated the reduction percentage of shoot and root growth (Table 1). The results properly show the influence of EtBr on plants. However, the roots of alfalfa and tomato`s root had the maximum and minimum sensitivity, respectively. Interestingly, the highest concentration of EtBr decreased the shoot and root of both plants but it seems alfalfa was more affected. In the other hand, tomato roots reduction percentages not only remarkable but also they were not significant. In contrast to alfalfa`s growth (root and shoot) which were showed a slight decreasing rate from lowest to highest concentrations tomato`s roots and shoots did not have a meaningful differences. They were almost had a same reduction percentages except the plants which were inoculated by 154 mg/ml concentration of EtBr.

DISCUSSION

The best chosen plant for phytoremediation needs two main potent. First, the desired plant must produce sufficient biomass which could directly affect the observed chemical concentration. Second, such plants need to responsive to agricultural practice to repeat planting and harvesting the samples [12]. It is interesting that if the hazardous accumulate in the shoots, it is preferable than roots. If the chemicals can concentrated in the roots we should need to remove whole the plant but in the first one just removing the shoots could effectively solve the problem. The common pollutant accumulating plants according the several researches are seapink thrift, rayweed, Indian mustard, sunflower, wheat, corn and etc [13]. Our results added two tomato and alfalfa to this list. Of course, it seems to tomato will be better candidates than alfalfa. Due to EtBr`s unique structure, it can only intercalate into DNA strands [5]. Therefore, it is commonly used as nucleic acid fluorescent tag in various techniques of the life science field. From the first time of its use, 1950s, for veterinary treatment (cattle), the mutagenecity of them was clear. But, carcinogenicity or teratogenecity aspects still have to be well clarified. According to the reports, LD50s of EtBr are 1503 mg/kg in oral form and 34 mg/m^3 in halation per hour for rats. The scientists believed that necessarily the any solution with >0.15% EtBr had to be considered hazardous waste and dispose them through filtering with EH & SOR filters [5]. Additionally, Zollinger and Morais in 1979 showed the decrease in growth rate of infected chick embryo cells and synthesis inhibition of mitochondrial macromolecules by EtBr [14]. Additionally, the hazardous effects of EtBr on plants also were reported by Uera [6]. According such reports our high concentration (154 mg/ml) is 10 fold less than deadly concentration for rats and the results show 31% (shoot), 36% (roots) reduction percentage in alfalfa and 28% (roots) for tomato. It seems that the toxic effect of EtBr for plants is so lower than its toxicity for animals. But, we find out that the accumulation of EtBr on plants did not kill them and instead reduce the growth rate in contrast to controls. This reduction can refer to different phenomena, the lost of essential minerals in plants or the structural interference of EtBr with different activity of the plant cells. The essential minerals in plant could be lost just in the comparative absorbance of them in contrast to EtBr. The

Table 1: Reduction percentage of shoots and roots in test plants.

Concentration (mg/ml)	Alfalfa (R%)		Tomato (R%)	
	Shoot	Root	Shoot	Root
154	31	36	28	4
113	19	22	17	6
64	10	15	16	0
42	8	10	15	5
4.4	6	5	14	0

The reduction percentage of growth in root and shoot of two alfalfa and tomato were calculated by %R=(1-A/B)×100 in which A is the length of test shoot or root and B is the length of control shoot or root.

determination of plants that can work most effectively in a given application is the most important part in phytoremediation. A good candidate must grow quickly and consume large quantities of water in a short time. The tolerance of plants to chemicals may be related to the ability of plants to detoxify them and depends on many plant cellular properties. Tomato is a plant with phytoremediation properties [6] and the salt-drought tolerance of alfalfa make them efficient EtBr accumulator plants. Tomato was known not only as a delicious food but also used as a source of valuable medicine since the beginning of human civilization. Of course, alfalfa is one of functional crops around the world. The popularity of such crops will be make them cheap and valuable phytoremediation tools but in the other hand the hazardous effect of such agents could influence the live of human. The obtained results were confirmed this observations. It looks like that if tomato and alfalfa expose to EtBr, they will absorb it in a short time and interestingly not only it did not stop growth of them but also it was strong stimulator for the growth, but in the long term it could threat plants life.

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