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Test of combined bioremediation by *Hédysarum pallidum* and *Aspergillus tubengensis* of antimony contaminated soil.

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***Abstract*- The main objective of this study was to examine the ability of inoculation with an endomycorrhizal fungus *Aspergillus tubengensis* to increase the efficiency of phytoremediation by an antimony-accumulating plant *Hedysarum pallidum*, and to study the oxidative responses of this combination. To do this, a combined remediation trial was carried out in the greenhouse for the first time, using two types of *Hedysarum pallidum* seed from different sources, one from a polluted area and the other from an unpolluted area, inoculated with *Aspegillus tubengensis* in increasing concentrations of Sb 5, 20, 50 and 200 ppm. After 45 days of testing, proline, soluble sugars, MDA, proteins, SOD and catalase were measured by spectrometry. The results showed that inoculation with a fungus significantly increased the accumulation of proline and soluble sugars ($p < 0.001$). MDA increased significantly as a function of Sb concentration and inoculation ($p < 0.001$). Proteins increased significantly as a function of inoculation ($p < 0.001$) but not significantly as a function of Sb ($p > 0.05$). Oxidative stress introduced a significant increase ($p < 0.001$) in SOD and catalase. These antioxidant stress markers revealed that seeds of polluted origin developed resistance to metal stress. The mycorrhization association between *Hedysarum pallidum* and *Aspergillus tubengensis* is a better means of successfully remediating antimony-contaminated soils.**
***Keywords*— bioremediation, *Hédysarum pallidum*, *Aspergillus tubengensis*, inoculation, antimony.**

Introduction

Soils contaminated by metals have a serious environmental and health problem over the years. One of these metals, antimony which is a non-essential element for plants [1] considered as a potentially toxic element [2]. Several methods are used for the removal of heavy metals but they are expensive [3]. Phytoremediation is an emerging technology using plants to remediate soil contaminated by heavy metals [4]. *Hedysarum pallidum* Desf is an antimony accumulator species [5]. The use of fungus can be a good bioremediation alternative [6]. Mycorrhizal fungus plays a role in phytoremediation [7]. The idea of using mycorrhizal fungus to improve phytoremediation has been proposed for several years [7] [8]. Several authors demonstrate the correlation between fungi such as Aspergillus and their ability to eliminate metals [9]. Antimony-resistant endomycorrhizal fungus *Aspergillus tubengensis* [10] was used in this test for its tolerance to antimony.

Materials and Methods

A. *Plant material*

The seeds used in this trial came from two sources (a contaminated area and an uncontaminated area) in the Ain Bebouche region in eastern Algeria. After undergoing heat shock, the seeds were surface sterilized using 2% sodium hypochlorite for 3 minutes, rinsed with sterile distilled water and left to pregerminated in petri dishes). After 15 days, the young seedlings are transplanted into pots in a greenhouse.

B. *Fungal material*

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The fungal strain used for inoculation *Aspergillus tubengensis*, isolated from the roots of *Hedysarum* The strain is subcultured on a Sabouraud medium and incubated for 15 days at 25ºC.

The substrate used is (a potting soil) contaminated with antimony (antimony tartrate $C_8H_4K_2O_{12}Sb_2$, $3H_2O$), with the following concentrations: 5ppm, 20ppm, 50ppm, 200ppm.

C. *Inoculation*

Using the method described by [11] the seedlings were water-stressed for 3 days prior to inoculation. They were then carefully dug up and the roots were washed to remove excess culture substrate. The root tips were cut with sterilized scissors. The roots were then immersed in 10 ml of inoculum for each plant for 30 minutes.

D. *Experimental design*

For each type of seed (two compartments) (GP, GNP) different antimony treatments were carried out: 5ppm, 25ppm, 50ppm, 200 ppm, so each treatment contains a sub-treatment: one plant inoculated with *Aspergillus tubengensis* (I), the other a non-inoculated plant (NI), each sub-treatment comprises 3 plants per pot. The control for each compartment consisted of an uncontaminated, non-inoculated negative control. In addition, 5 replicates were made for each observation.

E. *Parameters mesure*

The levels of proline, soluble sugars, MDA, COD and CAT were quantified. Proline was determined using the method of Monneveux and Nemmar (1986), soluble sugars were determined using the method of Dubois (1956), and malondialdehyde MDA was quantified using the method cited by [5], to assess oxidative damage to lipids, protein concentration was calculated according to the method of 12 using BSA (bovine serum albumin) as the standard, the total activity of SOD was evaluated according to the method of 13, (CAT) was measured according to the method of 14.

F. *Statistical analysis*

The obtained data for the different tested biomarkers at increased Sb concentrations were presented in bar graphs and correspond to means (± SEM) of 3 repetitions. The significance of the differences between the means, at the level of $p < 0.05$, was estimated using a variance analysis (ANOVA) followed by the Tukey's test.

RESULTATS AND DISCUSSION.

Accumulation of proline and soluble sugars

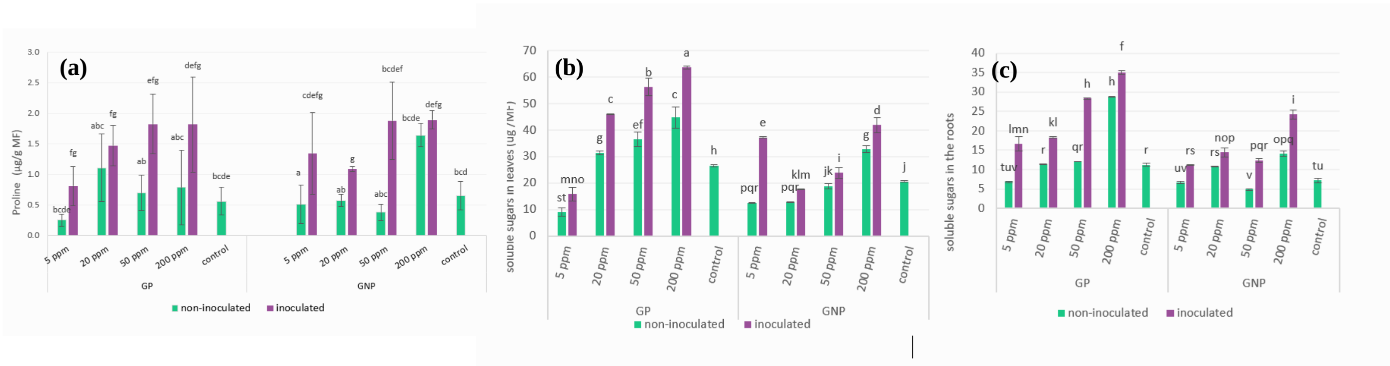

Figure 1: Biochemical response of *Hedysarum pallidum* inoculated by *Aspergillus tubengensis* in differente concentrations of antimony. (a): proline content, (b)sugar content in leaf, ((c) sugar content in roots. GP: polluted seeds, GNP: unpolluted seeds. Values represent means ± SEM (N = 3).

Proline levels increased significantly at the highest Sb concentration of 200 ppm in non-inoculated plants, and decreased at the lowest antimony concentration of 5 ppm. Statistical analysis using a 3-factor ANOVA showed that the effect of seed origin was non-significant for proline levels with ($p > 0.05$). While the effect of

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the concentrations of the pollutant Sb was significant with ($p < 0.05$). In contrast, the inoculation effect was highly significant for proline levels in Hedysarum with ($p < 0.001$).

The results obtained are consistent with those obtained by [15], who reported a significant accumulation of proline in plants exposed to antimony, which reduces the toxicity of Sb. Our results suggest that the use of the endomycorrhizal fungus *Aspergillus* promotes proline accumulation, which stimulates the plant's defense mechanisms in the face of stress. Soluble sugar levels accumulate strongly after the application of metallic stress and inoculation. Seeds of polluted origin accumulated more soluble sugars than seeds of unpolluted origin. The analysis of variance showed significant values for all factors with ($p < 0.001$) which means that the origin of the seeds and the inoculation and treatment have a very significant impact on the accumulation of sugars indicating a physiological response of the plants. The results obtained show that a strong accumulation of sugars is recorded in high Sb concentrations which is in agreement with the results obtained by [16], on the response of *Atriplex halimus* to cadmium stress. Similar results were obtained in the faba bean Vicia faba on high lead concentrations [17] Soluble sugars are involved in the defence of chlorophylls and carotenoids against metal stress [18].

MDA

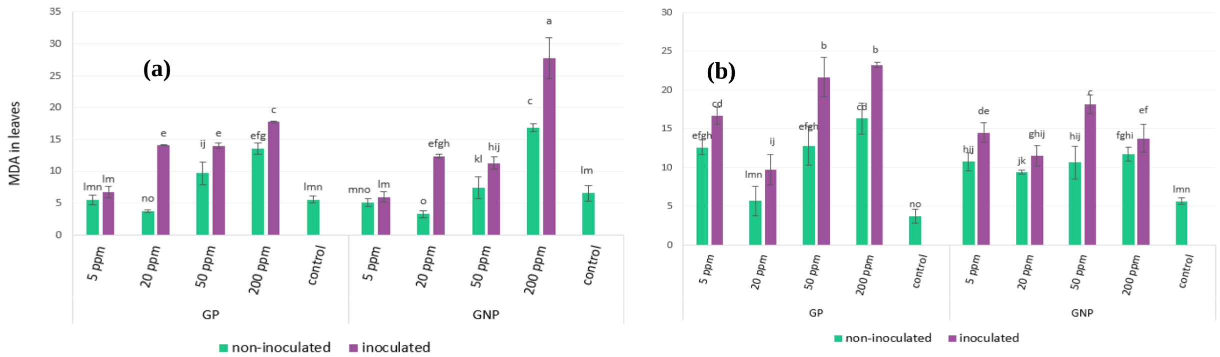

Figure 2: MDA content in *Hedysarum pallidum* inoculated by *Aspergillus tubengensis* in different concentrations of antimony. (a): MDA in leaves, (b) MDA in roots. GP: polluted seeds, GNP: unpolluted seeds. Values represent means ± SEM (N = 3).

A significant increase in MDA content as a function of Sb concentration and inoculation with *Aspergillus tubengensis*. These values are very high in the 50 and 200 ppm concentrations and decrease in the 5 and 20 ppm concentrations in the leaves. The MDA content was significantly higher in the GPs (seeds of polluted origin) than in the unpolluted seeds and then in the control, with the exception of the 200ppm Sb concentration, where the leaves showed the highest levels. These values increased significantly in inoculated subjects compared with non-inoculated subjects. Our results are in agreement with those obtained by [5] which indicate a strong increase in MDA in *Hedysarum pallidum* at high Sb concentrations in a mining area. This increase in MDA could be attributed to the increased concentration of polyunsaturated fatty acids compared to saturated fatty acids. ROS attack these unsaturated fatty acids and form lipid peroxides. [19]

Proteins

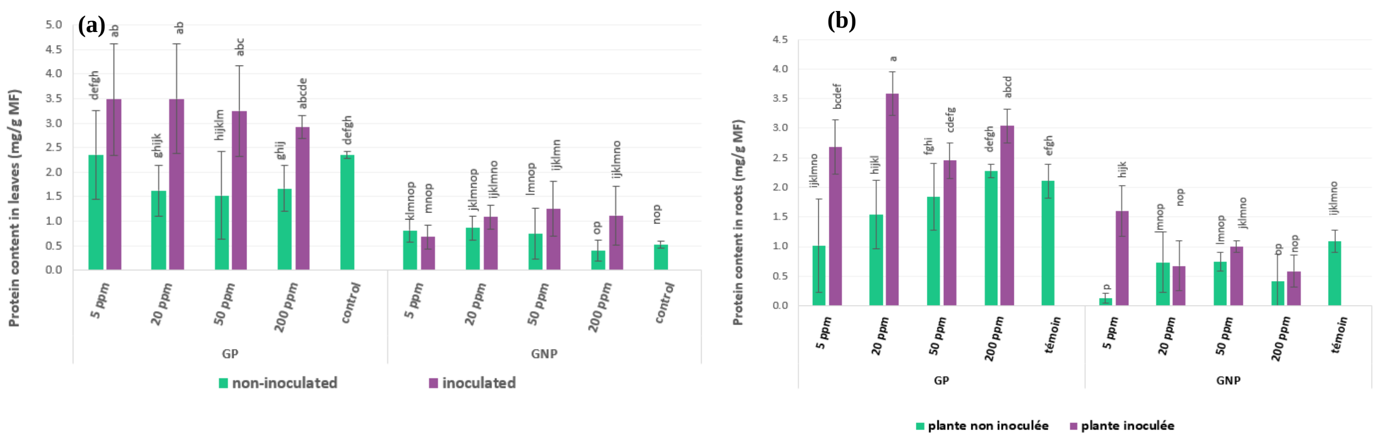

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Figure 3: Protein content in Hedysarum pallidum inoculated by Aspergillus tubengensis in different concentrations of antimony. (a): Protein in leaves, (b) Protein in roots. GP: polluted seeds, GNP: unpolluted seeds. Values represent means ± SEM (N = 3).

Analysis of variance showed a non-significant effect of Sb concentrations and the origin of the seeds and the plant part on protein content with ($p > 0.05$). However, the inoculation effect was highly significant ($p < 0.001$), suggesting that fungal inoculation substantially modifies plant responses. [20] indicates a dose-dependent decrease in protein in faba bean under lead stress.

Several authors attribute the decrease in proteins that causes the decrease in the protein pool to lipid peroxidation and the fragmentation of proteins under the action of ROS, probably as a result of over-activation of the proteases responsible for protein solution [21] [22].

SOD et CAT

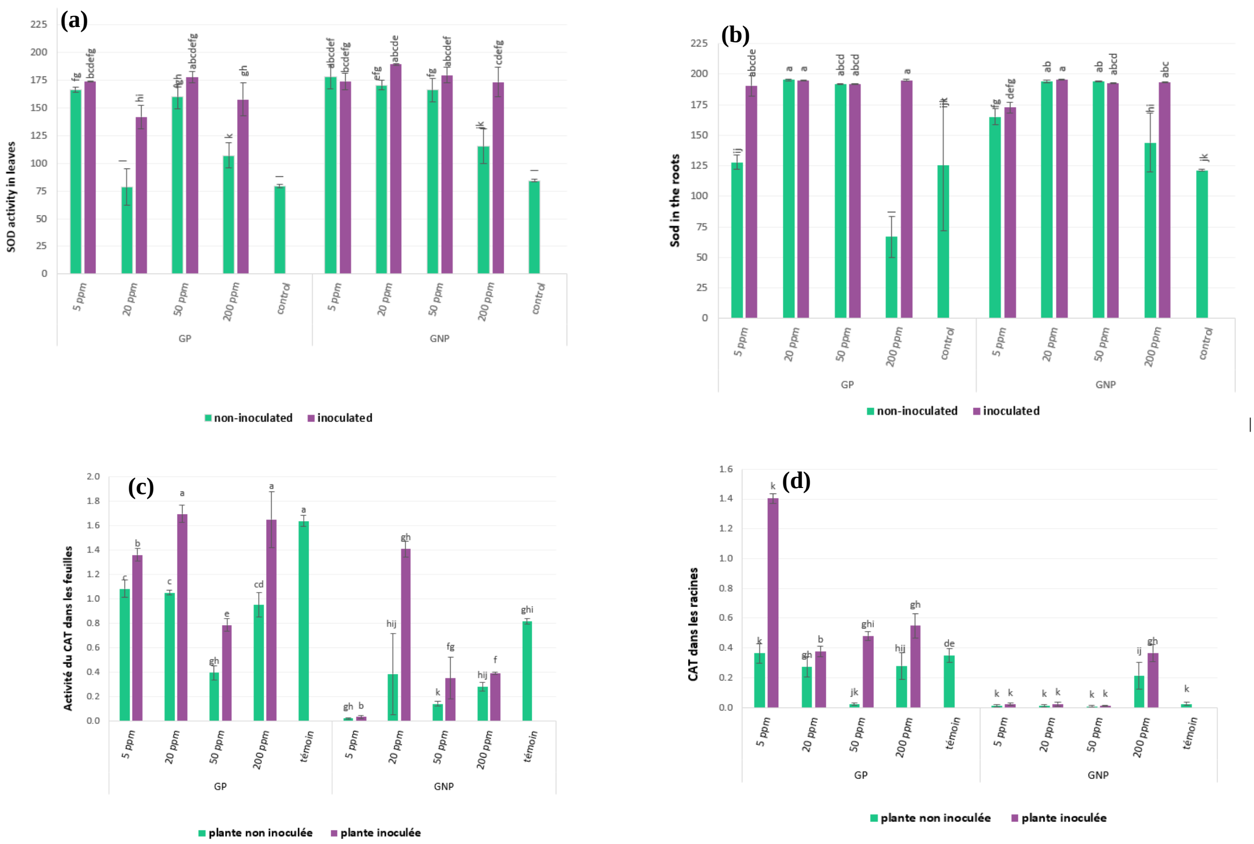

Figure 4: SOD, CAT content in *Hedysarum pallidum* inoculated by *Aspergillus tubengensis* in different concentrations of antimony. (a): SOD in leaves, (b) SOD in roots, (c): CAT in leaves, (d): CAT in roots. GP: polluted seeds, GNP: unpolluted seeds. Values represent means ± SEM (N = 3).

The action of this enzyme (SOD) increased significantly in the various Sb concentrations except for the 200ppm concentration in the roots of polluted seeds, where it decreased compared with the control. This value maintains the same kinetics in all parts of the plant. SOD was correlated and highly significant with inoculation, seed origin, Sb dose and plant part. These results corroborate those obtained by [5] where a significant increase in SOD is recorded as a function of antimony doses in this same steppe species. Also, [10], reported that exposure of *Aspergillus tubengensis* to antimony causes an increase in this anzyme. The increase in SOD in *Hedysarum pallidum* is consistent with the results of [23] who showed a linear increase in the values of this enzyme in Oryza sativa L rice with increasing doses of Sb.

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However, plants from polluted seeds behaved differently and catalase activity decreased at concentrations of 20, 50 and 200ppm. In fact, inoculated subjects showed a significant increase in catalase compared with non-inoculated subjects. Catalase is a very important enzyme in the system for degrading hydrogen oxide and detoxifying ROS in response to abiotic stress by catalysing H_2O_2 to H_2O in peroxisomes [24].

CONCLUSION

The aim of this research is to highlight the accumulative capacity of *Hedysarum pallidum* and the great resistance of the fungus *Aspergillus tubengensis* for effective and successful remediation of antimony-polluted soils.

Under semi-controlled conditions in the greenhouse, the remediation coupling *Hedysarum pallidum* and *Aspergillus tubengensis* by symbiosis, for the first time in Algeria, shows a good ability to remediate antimonial soils. This was shown by the results obtained, which indicate that inoculation increases the accumulation of proline and soluble sugars and MDA, which reduces the toxicity of Sb and stimulates the plant's defense mechanisms. The antioxidant responses of *Hedysarum pallidum* translated into increased SOD and CAT in the inoculated plants, an indicator of the success of the combined remediation.

The results indicate that polluted seeds accumulate more than unpolluted seeds, thanks to resistance in the plant cells or adaptation to extreme conditions. The results therefore indicate that the use of mycorrhizal fungi in symbiosis with accumulator plants is a promising biotechnology for the rehabilitation of antimony-contaminated soils.

REFERENCES

[1] S. Lafond, “Développement d'un procédé de traitement des sols d'une butte d'arrêt de tir à l'arme légère contaminés à l'antimoine, au cuivre, au plomb et au zinc,” Thèse Philosophiae Doctor (Ph.D.) en sciences de l'eau. Université Québec 2012.

[2] S. Diquattro, P. Castaldi, S. Ritch, A. L. Juhasz, G. Brunetti, K. G. Scheckel, G. Garau, E. Lombi “Insights into the fate of antimony (Sb) in contaminated soils: Ageing influence on Sb mobility, bioavailability, bioaccessibility and speciation,” Sci Total Environ. 2022. http://doi:10.1016/j.scitotenv. 2021.145354.

[3] Iskandar N L, Zainudin N A I M and Tan S G. Tolerance and biosorption of copper (Cu) and lead (Pb) by filamentous fungi isolated from a freshwater ecosystem. J. Environ Sci. 2011;23(5):824–830.

[4] S. Ashraf, Q. Alia,b, Z. A. Zahira, S. Ashrafa, H. N. Asghar, “Phytoremediation: Environmentally sustainable way for reclamation of heavy metal polluted soils,”. Ecotoxicology and Environmental Safety. 2019. https://doi.org/10.1016/j.ecoenv. 2019.02.068.

[5] A. Benhamedi., A. Bentellis, O. Rached, G. Du Laing, A. Mechakra, “Effects of antimony and arsenic on antioxidant enzyme activities of two Steppic plant species in an old antimony mining area,” Biol Trace Elem Res 158:96–104. https://doi.org/10.1007/s12011-014-9917-7. . 2014

[6] Khan G, Kuek C, Chaudhry T M, Khoo CS, and Hayes WJ. Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation.Chemosphere. 2000;41:197– 207.

[7] AG. Khan, C. Kuek, TM. Chaudhry, CS. Khoo, WJ. Hayes, “Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation,” Chemosphere 41: 197-207. 2000.

[8] V Göhre, U Paszkowski, “Contribution of the arbuscular mycorrhizal symbiosis to heavy metal phytoremediation,” Planta 223: 1115-1122. 2006.

[9] Saba G, Thirumarimurugan M and Sivakumar VM. Heavy Metal Tolerance Potential of Fungus Isolated From Copper Smelting Industry. Int. Res. J. Pharm. 2017; 8 (6): 120–125.

[10] O. Meghnous, L. Dehimat, P. Doumas, M. Kassa-Laouar, F. Mosbah, O. Rached, “Oxidative and antioxidative responses to antimony stress by endophytic fungus Aspergillus tubingensis isolated from antimony accumulator Hedysarum pallidum Desf,” Biologia. 2019. https://doi.org/10.2478/s11756-019-00305-z.

[11] Bayaa, Erskin w, Hamedi A. Evaluation of a wild lentil collection for resistance to vascular genetic ressources and crop evolution. 1995: 231-235.

ISSN: 1737-9334

[12] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275.
[13] Marklund S, Marklund G (1974) Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 47:469–474. https://doi.org/10.1111/j.1432-1033.1974.tb03714.x.
[14] Chance B, Maehly AC (1955) Assay of catalases and peroxidases. Methods Enzymol 2:764–775. https://doi.org/10.1002/9780470110171.ch14.

ISSN: 1737-9334