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PROSPECTING THE INFLUENCE OF POTTING SUBSTRATE AND AM INOCULATION ON *Iris pseudacorus* L.

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Abstract

Iris pseudacorus is an ornamental macrophyte with phytoremediation capacity and medicinal value. In this research, it was used as model plant for study of four commonly occurring micromycetes: arbuscular mycorrhizae (Glomeromycota), fine root endophytes (Mucoromycotina), dark septate endophytes (Ascomycota) and Olpidium sp. (Chytridiomycota). Experiment was established with two substrate types: peat and bark humus, and inoculation treatment with three graduations: 2 and 5 AMF species and non-inoculated respectively. Root samples were collected for microscopic analysis after 3 months in pots and open field. Results show that all AM colonization parameters were higher in field compared to pots, but influence of AM inoculation decreases in field due to established background soil microflora. Frequency of DSE and Olpidium sp. was much higher in pots. Bark humus had a lasting positive effect on plant development. Compared to field, pot growing media could be more prone to microbiome disbalance perhaps due to lack of stability in natural-occurring mechanisms that act to regulate complex interaction dynamics. Understanding conditioning relationship between soil micromycetes across contrasting growing conditions could help addressing practical challenges associated with use of microbial inoculants in agriculture.

Key words: *nutrient transfer, storage organ, micro-organism interaction, rhizosphere, microbiome stability.*

INTRODUCTION

Throughout time *Iris pseudacorus* also known as 'yellow flag' was attributed special meaning as well as practical uses. It was adopted as heraldic symbol by king Clovis I and several stories explain how he came to use this flower on his coat of arms after conversion to Christianity (Silverthorne, 2002; Giner-Sorolla, 2011). Evidence from archaeological excavations in Gdansk region indicates that flowers of *Iris pseudacorus* represented a source of dyes during XII-XIII centuries (Macchia et al., 2016). *Iris pseudacorus* was known also as medicinal plant in Europe (Crişan & Cantor, 2016), and rhizomes were used by English country people with syrup of buckthorn to treat dropsy (Frederick, 1821). Seeds of this plant can be used as coffee substitute (Engin et al., 1998). *Iris pseudacorus* still has practical applications today, because flowers are sources of colouring agents for cosmetic and food industry while rhizomes can provide natural dyes or components for ink preparation (Pippen, 2015; Crişan et al., 2018). *Iris pseudacorus* L. as an ornamental grows best in pond or bog gardens but can be introduced also in herbaceous border. Plant is characterized by yellow flowers, fibrous rhizomes pink in colour when sectioned. Both diploids and tetraploids are cultivated (White et al., 1997). There are cultivars with variegated foliage which can extend their ornamental contribution to landscape beyond spring bloom (Ondra, 2007). A consistent body of research is dedicated to phytoremediation capacity of *Iris pseudacorus*. Thus, studies proved potential of this species to treat urban wastewater (Zhang et al., 2017), to decontaminate water of certain agricultural pesticides (Wang et al., 2013) and heavy metals (Caldelas et al., 2005), or soils from petrochemical residues (Wang et al., 2016). Previous studies on arbuscular mycorrhizae in *Iris pseudacorus*, demonstrated that inoculation with species *Diversispora epigaea, Glomus aureum, Rhizophagus irregularis, Rhizophagus clarus,* enhanced plant tolerance to toxic metals in the environment (Wężowicz et al., 2015). Aim of this study was to prospect the influence of two commonly used potting substrates and AM inoculation with commercial products on *Iris*

pseudacorus plants. To achieve this, two objectives were considered:
- describing functional relationship between micromycetes in pot/field conditions with implications for soil-plant health balance;
- identification of inoculation persisting effect after transplanting in the field with implication for plant development and efficiency of inoculum application.

MATERIALS AND METHODS

The experiment was initiated in pots and continued in open field, after plants were transplanted (Figure 1). Starting biologic material of this study was represented by *Iris pseudacorus* shoots of similar size detached with roots from mother-rhizomes belonging to mature plants that overwintered in greenhouse. Plant material was washed with chlorine solution and had roots and longer leaves trimmed. Pot experiment was established in April 2018 and organized according to bifactorial design.
Factor A: unsterilized potting substrate with 2 levels - a_1 = mix of bog-peat decomposed medium-high, wood fibres, dolomite, perlite; a_2 = fermented bark humus, peat, perlite.
Factor B: AMF inoculum with 3 levels - b_1 = non-inoculated; b_2 = inoculated with commercial product containing 5 AMF species (*Funneliformis mosseae, Funneliformis geosporus, Claroideoglomus clarodeum, Rhizophagus intraradices, Glomus microaggregatum*); b_3 = inoculated with commercial product containing 2 AMF species *(Funneliformis mosseae, Rhizophagus intraradices)*. Calculation of dose application for each of the two inoculation products followed producer instructions. For both products AMF propagules were contained in an organo-mineral matrix.

Figure 1. *Iris pseudacorus* potted in two types of substrate, inoculated with two types of arbuscular mycorrhizae products and then transplanted in field (Original, 2018)

From the combination of the two factors resulted 6 experimental variants: V1 = peat + non-inoculated; V2 = bark humus + non-inoculated; V3 = peat + 5 AMF; V4 = bark humus + 5 AMF; V5 = peat + 2 AMF; V6 = bark humus + 2 AMF. Pots were kept outdoor on a porch to be sheltered from rain. Water was supplied by flooding system at few days interval. It was ensured that water could not travel from pots of one variant to another in order to prevent cross-transport of propagules or nutrients. No phytosanitary treatment was applied to plants. After 3 months in pots (July 2018), root samples were collected for microscopic analysis. Then, the plants had leaves trimmed and were transplanted together with whole root system and pot substrate in open field in Botanical Garden UASVM Cluj-Napoca, in randomized blocks. Because when removing weeds, the mycelia development in rhizosphere can be disturbed, mulch foil was applied prior to planting. Soil type in the garden is clay-loam with good NPK supply, low humus level and pH 6.7. After 3 months in the field (Oct. 2018), were conducted measurements for plant development and root samples were collected for microscopic analysis. Roots were prepared for microscopic observation following ink-vinegar staining method (Vidican & Stoian, 2016). Root colonization by arbuscular mycorrhizae *(Glomeromycota)* assessment was conducted according to method of Trouvelot et al. (1986) for 90 root segments per variant: 30 segments × 3 repetitions. A total number of 1080 root segments were assessed for 540 from potted plants and 540 from field, under Optika microscope at 100× - 400×. AM indicators

were calculated using Mycocalc software (https://www2.dijon.inra.fr/mychintec/).
In addition, were recorded observations for presence of other fungal colonizers (Figure 2): dark septate endophytes *(Ascomycota)*, fine root endophytes *(Mucoromycotina), Olpidium (Chytridiomycota)* for same root segments.

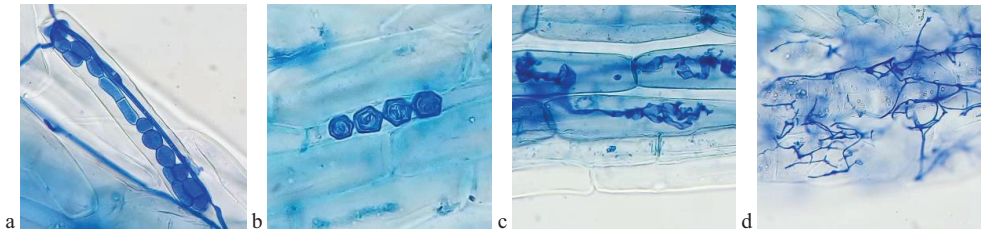

Figure 2. Micromycetes inside roots: a) *Ascomycota* – DSE*;* b) *Chytridiomycota – Olpidium;* c) *Glomeromycota* – AM*;* d) *Mucoromycotina* - FRE (Original, 2018)

Parameters subject to analysis were:
- F% = frequency of occurrence for AM, DSE, FRE, Olp. in roots;
- m% = intensity of the mycorrhizal colonization in the root fragments;
- M% = intensity of the mycorrhizal colonization in the root system;
- a% = arbuscule abundance in mycorrhizal parts of root fragments;
- A% = arbuscule abundance in the root system;
- plant height after 3 months in the field (6 months after initial inoculation in pots);
- number of leaves per plant after 3 months in the field (6 months after initial inoculation in pots).

Data analysis was conducted with Microsoft Excel 2016 and StatSoft Statistica 12.5.

RESULTS AND DISCUSSIONS

Predominant arbuscular mycorrhizae colonization observed both in pots and field corresponds to *Paris* morphotype. But compared to arbuscules from the field samples, in pot conditions intra-cellular coils often presented a swollen appearance. A few numbers of root segments from pots and field had arbuscules that showed an intermediate morphology or even *Arum*-like, but occurrence was very low. Comparing Table 1 and Table 2 can be observed that in pot conditions, arbuscular mycorrhizae parameters such as intensity in root fragments and arbuscularity both in root fragments as well as in root system is correlating significantly positive with experimental variants. By comparison, in field conditions for same parameters, there is no positive correlation. This comes to show that after 3 months in the open field and 6 months since initial inoculation in pots, the variant does not exercise a strong or significant influence anymore. This is more clearly illustrated by the fact that in pot conditions all colonization parameters correlate positively with inoculation, and significantly positive with intensity of colonization in root fragments. In field conditions between inoculation treatment and colonization parameters are no positive correlations found. Because, by this time plants have new roots and are being colonized also by fungi natural occurring in the soil and initial inoculation does not exercise as much of an influence. In addition, in pot conditions, there is noticeable a positive correlation between substrate type and all arbuscular mycorrhiza parameters, with significant positive coefficient for arbuscularity in root fragments. This indicates that this factor can affect the development of mycorrhiza and should be taken in consideration by farmers when choosing potting substrates with intend to also apply commercial AMF inoculum. In field conditions can be identified a significant correlation between frequency and intensity in root system while in pot conditions this was not the case. The explanation is that in pot conditions other factors exercised a strong influence, such as substrate type and abundance of other fungal endophytes resulting from weak balancing interaction. In field conditions, was also identified a significant positive correlation between intensity in mycorrhizal parts of root fragments and intensity in root system, and

arbuscularity in mycorrhizal part of root fragments and root system. Both in pots and field conditions can be observed a significant positive correlation between arbuscularity in mycorrhizal parts of root fragments and root system with nearly perfect coefficient in pot conditions.

Table 1. Correlation matrix for arbuscular mycorrhizae parameters in *Iris pseudacorus* from pots (July 2018)

Variables	Variant	Substrate	Inoculation	F%	m%	M%	a%	A%
Variant	-	0.293	**0.956**	0.178	**0.535**	0.424	**0.470**	**0.487**
Substrate	0.293	-	0.000	0.421	0.279	0.373	**0.468**	0.418
Inoculation	**0.956**	0.000	-	0.057	**0.475**	0.329	0.348	0.381
F%	0.178	0.421	0.057	-	0.211	-0.005	-0.051	-0.005
m%	**0.535**	0.279	**0.475**	0.211	-	-0.004	0.384	0.454
M%	0.424	0.373	0.329	-0.005	-0.004	-	**0.545**	**0.474**
a%	**0.470**	**0.468**	0.348	-0.051	0.384	**0.545**	-	**0.986**
A%	**0.487**	0.418	0.381	-0.005	0.454	**0.474**	**0.986**	-

Note: Bold Pearson coefficient values designate significant correlation between variables at $p < 0.05$

Table 2. Correlation matrix for arbuscular mycorrhizae parameters in *Iris pseudacorus* from field (Oct. 2018)

Variables	Variant	Inoculation	F%	m%	M%	a%	A%
Variant	-	**0.956**	0.066	-0.036	0.004	**-0.490**	-0.353
Inoculation	**0.956**	-	-0.110	-0.192	-0.190	-0.439	-0.416
F%	0.066	-0.110	-	0.382	**0.763**	0.076	0.394
m%	-0.036	-0.192	0.382	-	**0.835**	**0.491**	**0.734**
M%	0.004	-0.190	**0.763**	**0.835**	-	0.367	**0.765**
a%	**-0.490**	-0.439	0.076	**0.491**	0.367	-	**0.810**
A%	-0.353	-0.416	0.394	**0.734**	**0.765**	**0.810**	-

Note: Bold Pearson coefficient values designate significant correlation between variables at $p < 0.05$

In field conditions this coefficient is slightly lower in value perhaps because the soil particularities are influencing mycorrhiza spreading. This strong relationship between variables could hint to uniformity of nutrient exchange structures distribution across roots.

Also, both in pots and field conditions was identified a significant positive correlation coefficient between intensity in root system and arbuscularity in root system. In pot conditions, all plants grown on bark humus had a faster development in first three weeks. Only after about a month plants from peat substrate started to reach similar height. After 3 months in pots, plants had their leaves trimmed and were transplanted in the field. From Figure 3 can be seen that after 3 months in field plants belonging to non-inoculated bark humus pot substrate (V2) were the tallest and had highest number of leaves per plant. Second tallest plants corresponded to variant inoculated with 5 AMF species and grown on bark humus (V4), while second highest number of leaves was found in plants inoculated with 2 AMF species and grown also on bark humus substrate (V6). Shortest plants and with smallest number of leaves per plant were found for variant corresponding to non-inoculated plants grown previously in pots with peat (V1). Because carbon fixed by photosynthesis is the trade-off for arbuscular mycorrhizal fungi nutrient transfer to plants, it could be speculated that non-inoculated plants did not had to partition biomolecules between host metabolic activity and fungi at the same rate as inoculated plants, and thus plants grown in rich nutrient humus substrate had more resources available to carry an accelerated growth without an intense root colonization to drain carbon. In addition, potted plants grown in this nutrient rich substrate were probably able to accumulate more resources in the storage organ and after transplanting in field had the advantage of more nutrients available to be relocated in for plant growth

before the root system even adjusted well to new conditions, suggesting that this substrate brings important advantages for establishment of plants following transplantation.

Among variants, differences are smaller for plant height but larger for number of leaves per plant as seen in Figure 3. Assessment for 3 other categories of fungal root endophytes showed that in pot conditions their frequency can be strikingly different compared to field conditions.

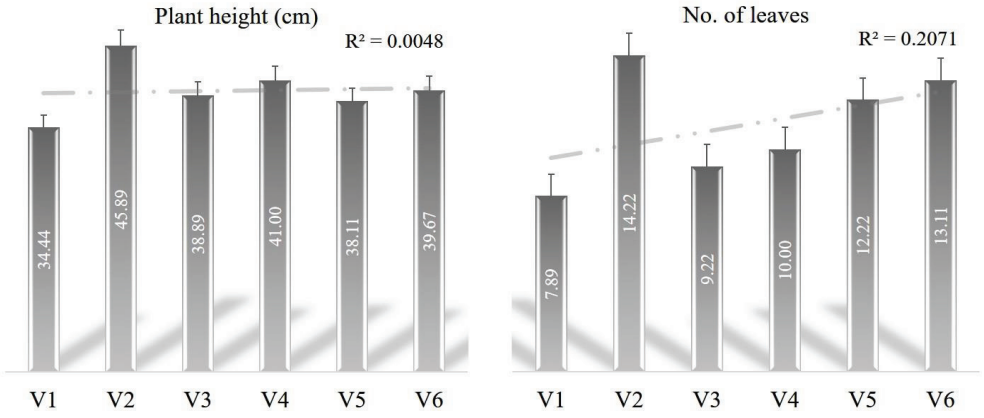

Figure 3. Average plant height and number of leaves per plant in *Iris pseudacorus* after 3 months in field (2018)

From Figure 4 can be observed that frequency of dark septate endophytes and *Olpidium* sp. was much higher in pots than in field conditions. Contrary, fine root endophyte is identified only in two variants in pots (V3, V6), while in field conditions occurs in all variants. However, both in pots and field FRE frequency maintains low. Results interpretation of this research is based on presumption that commercial inoculum used was free of potential contamination of propagules belonging to other fungal groups, such as DSE.

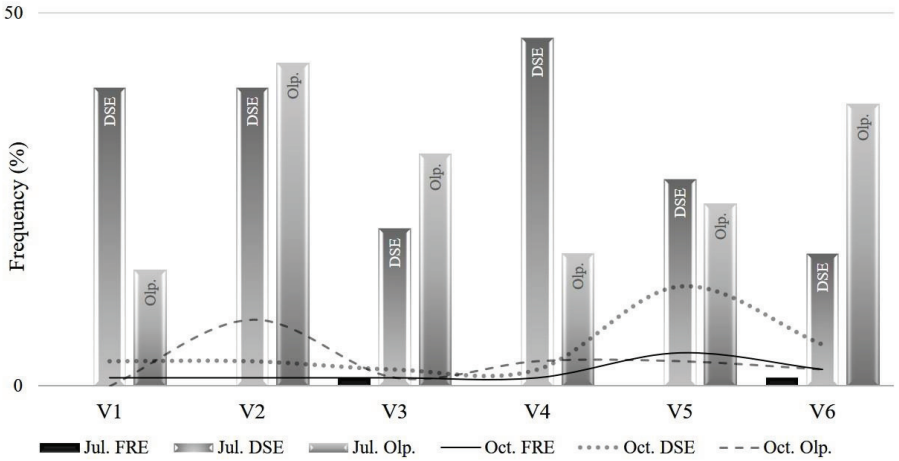

Figure 4. Comparative occurrence of FRE, DSE and *Olpidium* in *Iris pseudacorus*: after 3 months in pots (Jul. 2018), after 3 months in open field (Oct. 2018)

It can be clearly seen how in field conditions the distribution of all three fungal endophytes is balanced, and for neither of them frequency exceeded 15%. Due to the fact that no phytosanitary treatments were applied, the lower frequency of these can be associated with

a more complex soil microflora in the field. In field conditions, pathogen *Olpidium* sp. decreases in all variants but particularly in AM-inoculated ones (V3-V6). In pots, V2 presents highest frequency of *Olpidium* sp. and although in field conditions frequency decreases in value, it remains the highest among variants. It can be observed that inoculated plants from peat substrate (V3, V5) had a lower colonization by dark septate endophytes compared to plants non-inoculated grown also on peat (V1). This might indicate a negative interaction between *Glomeromycota* fungi and *Ascomycota* endophytes. When comparing average values for entire experiment corresponding to each endophytic parameter studied (Figure 5) it is evident that all AM colonization indicators were higher in field conditions than in pots: almost twice as high for frequency, and 3 to 4-times as high for intensity of AM colonization in mycorrhizal parts of root fragments and in root system respectively. DSE and *Olpidium* frequency in pot conditions reached average frequency of 32% and respectively 28%. In field conditions, for none of these two the overall average frequency exceeds 5%. This suggests two things. First, microflora in pot conditions could be more prone to disbalance lacking perhaps a certain degree of resilience required for resorting natural regulating mechanisms existing in field soil. Secondly, there could be a certain level of arbuscular mycorrhizae diversity required for a successful interaction with respect of plant rhizosphere microbiome in the sense that these would prevent other fungal endophytic groups to become overwhelming colonizers with detrimental implication for both success predictability of inoculation and intended effects.

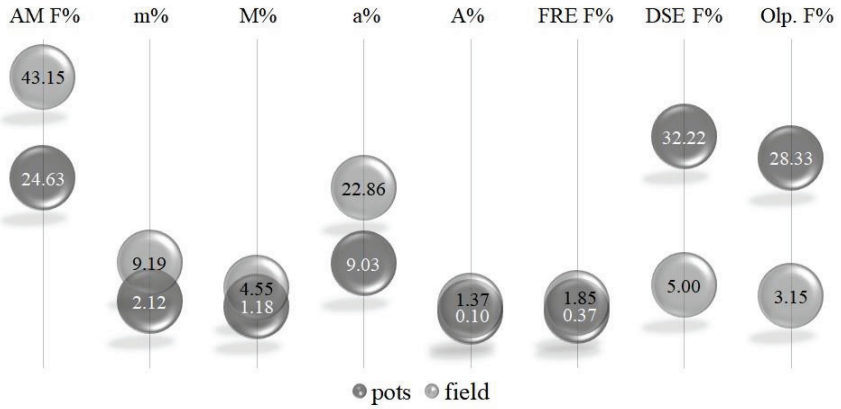

Figure 5. Experiment average values for studied parameters of AM, DSE, FRE., Olp. (2018)

Previous studies demonstrated positive influence of arbuscular mycorrhizae over vegetative characteristics in *Iris germanica* following supplementary inoculation in field conditions and similar pedo-climatic conditions (Crişan et al., 2017). Another study demonstrated that mycorrhization of *Iris* plants increased the absorbing rate of both nitrogen and phosphorous (Chen Y. et al, 2014) demonstrating that also for *Iris* plants, AM can have beneficial effects. Also, it seems that substrate particularities play a decisive role in rhizosphere microbiome stability and dynamics, because can exercise suppression of AM development with results hinting as concurring cause a biological component. Thus, ecosystem services of AMF depend to a large degree on the specific soil microbiome (Svenningsen et al., 2018).

In conclusion, increase of colonization by unintended fungi such as *Ascomycota* endophytes or pathogenic *Olpidium* sp. in pots, could be a result of weak interaction-based auto-regulation mechanism and unbalanced competition. In field conditions although the colonization is becoming equilibrated across fungal endophytes while arbuscular mycorrhizae develops better, the inoculation is not exerting a very strong influence due to background established community. These

might cause the effects of inoculation in field to be hard to distinguish while in pots there is the risk of being either in negative interaction with less-beneficial fungi or to be overrun by these, leading to a less satisfactory overall result. In future a better understanding of the relationship existing between soil micromycetes could help optimising the use of fungal inoculants.

CONCLUSIONS

Experiment average values for AM colonization parameters were higher in field conditions compared to pots while DSE and *Olpidium* frequency reached much higher levels in pots than in field.

Results suggest that some of the most commonly used pot substrates might exhibit proclivity to microbiome disbalance perhaps due to weakening or reduced complexity of natural-occurring mechanisms that act to regulate soil microflora interaction and dynamics in field, and in this case making the effects of AM inoculation less predictable.

FRE was detected only in two variants in pot conditions but was found in all six variants in field conditions, although in both cases the occurrence maintained at low levels.

Bark humus substrate had a lasting positive effect on plant development.

REFERENCES

Caldelas, C., Araus, J.L., Febrero, A., Bort, J. (2005). Accumulation and toxic effects of chromium and zinc in Iris pseudacorus L., *Acta Physiologiae Plantarum*, *34*, 1217–1228.

Chen, Y., Wang., L., Fang., M., Jiang, X. F., Dong, J. (2014). Role of arbuscular mycorrhizal fungi on iris, *Journal of Agricultural Resources and Environment*, *31*(3), 265–272.

Crişan, I., Cantor, M. (2016). New perspectives of medicinal properties and uses of *Iris* sp. *Hop and Medicinal Plants*, *24*(1-2), 24–36.

Crişan, I., Vidican, R., Stoian, V., Cantor, M. (2017). The effects of supplementary mycorrhization regarding some vegetative characteristics at *Iris germanica* L. *Scientific Papers. Agronomy Series*, *60*(1), 209–214.

Crişan, I., Vidican, R., Morea, A., Simea, Ş. (2018). Dyeing potential of linen fabric with Iris flower extracts. *ProEnvironment, 11*(36), 223–227.

Engin, A., Kandemir, N., Şenel., G., Özkan, M. (1998). An autecological study on *Iris pseudacorus* L. (*Iridaceae*), *Turkish Journal of Botany*, *22*, 335–340.

Frederick, G.S. (1821). *A Supplement to the Pharmacopoeia: Being a Treatise on Pharmacology in general*, London: S. Gosnell Printer.

Giner-Sorolla, H. (2011). *A Christian's Treasury of Trees and Plants*. WestBow Press, 38.

Silverthorne, E. (2002). *Legends and Lore of Texas Wildflowers*. Texas University Press, 73.

Macchia, A., Prestileo, F., Cagno, S. (eds.) (2016). YOCOCU 2014: *Professionals' experiences in cultural heritage conservation in America, Europe and Asia*. Cambridge Scholars Publishing, 107.

Ondra, N.J. (2007). *Foliage: Astonishing Color and Texture Beyond Flowers*, Storey Publishing, 219.

Pippen, L. (2015). *Laurie Pippen's all-natural colorants for cosmetic, culinary, and textile dyeing*, Eiram Publishing, 86.

Svenningsen, N.B, Watts-Williams, S.J., Joner, E.J., Battini, F., Efthymiou, A., Cruz-Paredes, C., Nybroe, O., Jakobsen, I. (2018). Suppression of the activity of arbuscular mycorrhizal fungi by the soil microbiota, *The ISME Journal*, *12*, 1296–1307.

Trouvelot, A., Kough, J.L., Gianinazzi-Pearson, V. (1986). Mesure du taux de mycorhization VA d'un système radiculaire. Recherche de méthodes d'estimation ayant une signification fonctionnelle. In: V. Gianinazzi-Pearson & S. Gianinazzi (Eds.) *Physiological and Genetical Aspects of Mycorrhizae*. INRA Press, 217–221.

Vidican, R., Stoian, V. (2016). *Microbiology-Practical works.* Cluj-Napoca: Ed. AcademicPres, 214.

Wang, Q., Yang, J., Li, C., Xiao, B., Que, X. (2013). Influence of initial pesticide concentrations in water on chlorpyrifos toxicity and removal by Iris pseudacorus, *Water Science and Technology*, *67*(9), 1908–1915.

Wang, Y.N., Cheng, L.J., Zhou, Q.X. (2016). Phytoremediation of petroleum contaminated soils with *Iris pseudacorus* L. and the metabolic analysis in roots, *Huan Jing Ke Xue*, *37*(4), 1531–1538.

Wężowicz, K., Turnau, K., Anielska, T., Zhebrak, I., Gołuszka, K., Błaszkowski, J., Rozpądek, P. (2015). Metal toxicity differently affects the Iris pseudacorus-arbuscular mycorrhiza fungi symbiosis in terrestrial and semi-aquatic habitats, *Environ Sci Pollut Res Int.*, *22*, 19400–19407.

White, B., Bowley, M., Brearley, C., Christiansen, H., Cohen, O., Davis, A.P.,… Waddick, J.W. (1997). *A Guide to Species - Irises, their identification and cultivation*, Cambridge University Press, 163.

Zhang, X.B., Liu, P., Yang, Y.S., Chen, W.R. (2017). Phytoremediation of urban wastewater by model wetlands with ornamental hydrophytes. *J. Environ Sci.*, *19*(8), 902–909.

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