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# Biostimulant based on *Inula viscosa* L. (*Dittrichia viscosa* L.), algae and microorganisms in the growth and defense of *Spinacia oleracea* L. and *Lactuca sativa* L.

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***Abstract-*** In this study the possibility of using a biostimulant based on *Inula viscosa* L. *(Dittrichia viscosa* L.*)* to improve the growth and quality of *Spinacia oleracea* L. and *Lactuca sativa* L. plants and protection against *Pythium spp.* was evaluated. *I. viscosa* plants play a role agro-ecological very important because: i) show high allelopathic activity as they produce chemical compounds toxic to other plant species; ii) act as a dwelling place for antagonists useful to combat plant pests and therefore fundamental in the biological struggle; iii) find application in the phyto-remediation of contaminated soil in processesn of phyto-extraction, bioaccumulation. The experiments, started in September 2020, were conducted in the greenhouses of CREA-OF in Pescia (Pt), Tuscany, Italy. The five experimental groups in cultivation were: i) group without biostimulant, irrigated with water and substrate previously fertilized; ii) group with microrganisms, irrigated with water and substrate previously fertilized; iii) group with algae, irrigated with water and substrate previously fertilized; iv) group with Inula viscose, irrigated with water and substrate previously fertilized; v) group with INORT: mix microorganisms + *Inula viscosa* + algae, irrigated with water and substrate previously fertilized. All *Lactuca sativa* L. and *Spinacia oleracea* L. plants treated with a biostimulant based on *Inula viscosa* showed a significant increase in the agronomic parameters analyzed compared to the untreated control. The results show that the use of the biostimulant introduced in the medium can improve the quality of treated plants by significantly increasing seed germination and plant growth. The data also show that the use of this product has effects on the protection of plants from *Pithyum spp.*

Therefore, further interesting and innovative aspects of the use of this plant have been highlighted, given its acknowledged importance from a medicinal, melliferous and biodiversity point of view. Further experiments are currently underway to confirm the use of INORT as a possible product that can guarantee the grower a possible reduction of fertilizers and plant protection in horticulture.

***Keywords-*** Microorganisms; Sustainable applications; Plant extract; Rhizosphere, Plant stimulation

## I. INTRODUCTION

The *Inula viscosa* L. (syn. *Dittrichia viscosa* Greuter) (family Compositae) is a perennial bushy plant, generally evergreen, the largest among the Phanerogams, which includes an estimated number of genera around 959, rather common in the Mediterranean regions [1] (Figure 1). The I. viscosa is a perennial shrub plant, with a characteristic smell, which has erect cauli, woody at the base, richly covered with leaves, with a height of between 50 and 150 cm. The leaves, linear-lanceolate, are rough and green with the upper part richly covered with hairs and glands. The pyramidal inflorescence is composed of numerous flower heads with golden yellow flowers. Flowering takes place in autumn, while the fruits are made up of achenes. This plant has long been the subject of studies in relation to the presence in different parts of the plant (roots, stem, leaves, flowers, etc..) of numerous components of different chemical nature, whose properties make interesting some potential applications in pharmaceutical, cosmetic, aromatic, aromatic and related [2]. In fact, this species has long been known for its properties and therefore used in folk medicine essentially to treat liver disorders, such as analgesic, anti-inflammatory, antipyretic, antipyretic, anthelmintic and antifungal [3,4].

It is defined by several authors as "typical plant of the Mediterranean regions" [5,6,7] in fact, it is present in southern Europe, in North Africa, but there are no precise habitats of growth can be distinguished. It is very easy to recognize it in rural environments and near roadsides, prefers a calcareous and clayey soil, many times acidic and being a heliophilous species is possible to find it from 0 to 800 m a.s.l.. For its wide distribution is considered to be a pest and for several years no scientific studies have been carried out on its potential but, in recent years, it has been widely revalued [8].

*I. viscosa* is an important source of livelihood for butterfly caterpillars and moths. [9,10]. In addition, it is recognized for the its high melliferous power as it is a plant abundantly foraged by bees. Inula has a very long flowering period between late spring and early autumn, when most plants are generally no longer available in bloom. Therefore, the classroom has a high usefulness for the establishment of strong families of insects, to the aim to produce consistent swarms in the following spring. This plant also contributes to the production of honey in late summer and autumn [11,12].

## II. RELATED WORK

Numerous studies have been conducted to identify and isolate active compounds from Inula biomass. Among the most important classes of chemical compounds present within the Inula are mainly mono-, sesqui- and tri-terpenes, flavones, flavanones and carbohydrates. Both organic extracts and some metabolites showed antifungal activity towards dermatophytes and Candida albicans [13], antioxidant properties and anti-inflammatory effects in rats [14], fungicidal activity towards some pathogens of agricultural interest [15,16]. Studies on I. viscose extracts have shown control capacity against pathogenic fungi while secondary plant metabolites seem to show insecticidal and antibacterial capacity [14,16]. As a result of several studies it has been demonstrated that I. viscose plants play a role agro-ecological very important because: i) show high allelopathic activity as they produce chemical compounds toxic to other plant species; ii) act as a dwelling place for antagonists useful to combat plant pests and therefore fundamental in the biological struggle; iii) also find application in the phyto-remediation of contaminated soil in processesn of phyto-extraction [17,18], bioaccumulation [19] and as bioindicator [20]. These activities are closely related to the phenological phases of the plant.

Experimental evidence shows that Inula extract can be used in the control of varroa and nosema in bees, introduced into gel blocks and exploiting olfactory disorientation [21,22,23].
In this study the possibility of using a biostimulant based on *Inula viscosa* L. *(Dittrichia viscosa* L.*)* (Figure 1) to improve the growth and quality of *Spinacia oleracea* L. and *Lactuca sativa* L. (Figure 2) plants and protection against *Pythium spp.* was evaluated.

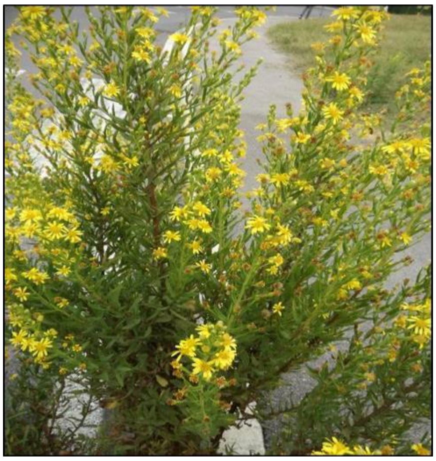

Figure 1 – Details of *Inula viscosa* plants

## III. METHODOLOGY

The experiments, started in September 2020, were conducted in the greenhouses of CREA-OF in Pescia (Pt), on *Lactuca sativa L. and Spinacia oleracea L.* plants.
The plants were grown in pots with a diameter of 12 cm.; 30 rooted cuttings per thesis, divided into 3 replicas of 10 plants each. All plants were fertilized with slow release fertilizer (2 kg $m^{-3}$ Osmocote Pro® for 6 months) introduced into the growing medium at the time of transplanting.
The five experimental groups in cultivation were:

- Group without biostimulant (CTRL): (peat 70% + pumice 20%), irrigated with water and substrate previously fertilized;
- Group with microrganisms (MC): (peat 70% + pumice 20%), irrigated with water and substrate previously fertilized, (Lactic acid bacteria, Photosynthetic bacteria, Yeast, Arbuscular mycorrhizae: 4 x $10^3$ spores/ml) 1% per week during the growing cycle (25 ml per plant);
- Group with algae (AG): (peat 70% + pumice 20%), irrigated with water and substrate previously fertilized, 1% per week during the growing cycle (25 ml per plant);
- Group with *Inula viscosa* (INU): (peat 70% + pumice 20%), irrigated with water and substrate previously fertilized, 1% per week during the growing cycle (25 ml per plant);
- Group with (INORT) (mix Microorganisms + *Inula viscosa* + Algae): (peat 70% + pumice 20%), irrigated with water and substrate previously fertilized, 1% per week during the growing cycle (25 ml per plant). The product INORT was supplied by the Francesco Attanasio farm.

The plants were watered 2 times per day and grown for 3 months. The plants were irrigated with drip irrigation. The irrigation was activated by a timer whose program was adjusted weekly according to climatic conditions and the fraction of leaching. On November 9, 2020, percentage of seed emergence, total leaf area per plant ($mm^2$), primary root lenght (mm), number of leaves per plant, number of lateral roots, aerial part and root system (mg), were recorded. Additionally, in the experiment the presence of plant mortality following attacks of *Pithyum spp.* was evaluated.


Figure 2 – Details of *Lactuca sativa (A) and Spinacia oleracea (B) in greenhouse*

 

**Statistics**

The experiment was carried out in a randomized complete block design. Collected data were analysed by one-way ANOVA, using GLM univariate procedure, to assess significant ($P \leq 0.05$, 0.01 and 0.001) differences among treatments. Mean values were then separated by LSD multiple-range test ($P = 0.05$). Statistics and graphics were supported by the programs Costat (version 6.451) and Excel (Office 2010).

## IV. RESULTS AND DISCUSSION

All *Lactuca sativa* L. and *Spinacia oleracea* L. plants treated with a biostimulant based on Inula viscose showed a significant increase in the agronomic parameters analyzed compared to the untreated control (CTRL). The results show that the use of the biostimulant introduced in the medium can improve the quality of treated plants by significantly increasing seed germination and plant growth. The data also show that the use of this product has effects on the protection of plants from *Pithyum spp.*

In *Lactuca sativa* L., (Table 1) the seed germination rate was significantly higher in (INORT) with 73.13%, preceded by (INU) 68.98%, (AG) 68.78%, (MC) 67.87% and finally the untreated control with 59.79% (Figure 3). Regarding foliar area per plant (FA), the thesis that showed better results was (INORT) with 234.10 $mm^2$, followed by (INU) with 228.58 $mm^2$, (MC) with 228.42 $mm^2$, (AG) 226.40 $mm^2$ and lastly (CTRL) with 219.64 $mm^2$. Even for the roots lenght, (INORT) was the best thesis with 94.29 mm, replaced by (AG) with 90.62 mm, (INU) with 88.98 mm, (MC) with 88.47 mm and the control with 80.94 mm. Concerning the number of leaves per plant, (INORT) was again the best thesis with 14.00 leaves, preceded by (INU) with 12.00, (AG) with 11.60, (MC) with 11.00 and finally the control with 7.40 leaves. Same trend for number of lateral roots where (INORT) was the best thesis with 9.40, followed by (AG) 8.20, (MC) 7.22, (INU) 7.05 and last the control with 4.63. For the vegetative weight INORT was also the best thesis with 843.69 mg, closely followed by (AG) with 840.27 mg, (INU) with 839.28 mg, (MC) with 837.84 mg and then the control untreated with 832.20 mg (Figure 4B,4D). On the radical weight (INORT) was the best thesis with 50.72 mg, compared to (AG) with 49.06 mg, (INU) with 48.87 mg, (MC) with 46.84 mg and the untreated control with 42.96 mg (Figure 5B).

In *Spinacia oleracea*, (Table 1) the percentage of seed germination was significantly higher in (INORT) with 74.49%, followed by (AG) 70.27%, (INU) 69.96%, (MC) 68.15% and finally the untreated control with 64.04%. As regards the foliar area per plant (FA), the thesis that showed better results was (INORT) with 227.53 $mm^2$, then ( INU) with 221.68 $mm^2$, (AG) with 220.88 $mm^2$, (MC) 218.89 $mm^2$ and ultimately (CTRL) with 213.18 $mm^2$. For roots lenght, (INORT) was also the best thesis with 92.77 mm, followed by (INU) with 86.46 mm, (AG) with 86.42 mm, (MC) with 85.36 mm and then the control with 80.80 mm. Regarding the leaves per plant, (INORT) was again the best thesis with 11.61 leaves, followed by (AG) with 8.63, (INU) with 8.41, (MC) with 8.04 and finally the control with 6.64 leaves. Same trend for number of lateral roots where (INORT) was the best thesis with 9.43, followed by (AG) 7.41, (MC) 6.63, (INU) 6.42 and finally the control with 5.04. Even for the vegetative weight INORT was the significantly better thesis with 79.34 mg, followed by (AG) with 76.22 mg, (INU) with 75.38 mg, (MC) with 74.34 mg and the untreated control with 69.30 mg (Figure 4A,4C). For the root weight (INORT) was the best thesis with 37.27 mg, compared to (AG) with 34.88 mg, (INU) with 34.21 mg, (MC) with 33.26 mg and the untreated control with 30.36 mg (Figure 5A).

The test also showed that INORT biostimulant can also have an effect in the containment of the plants attack caused by the pathogen *Pythium spp.* in *Lactuca sativa* L. and *Spinacia oleracea* L.

In particular on *Lactuca sativa*, INORT was the best thesis with only 0.82 plants affected by the pathogen, (MC) 1.61, (INU) 1.83, (AG) 3.05 and finally the control with 3.44 plants affected by *Pythium spp*. In *Spinacia oleracea*, the INORT thesis also showed better results than the others with only 1.20 plants affected by *Pythium spp.*, followed by (MC) with 2.28, (AG) with 3.43, (INU) with 3.46 and the control (CTRL) with 3.81.

*Inula viscosa* is a typically heliophilous and ruderal species, which is easily found in wastelands, ruins, along roads and headlands, cliffs and escarpments. Due to its rusticity and adaptability, it also colonizes poor and dry, stony soils. It resists mowing vigorously and is rejected by livestock due to the stickiness of the leaves and the resinous aroma. It can become invasive behaving as a weed in degraded pastures and in the rows of extensive tree plantations (vineyards, olive groves, orchards), while eschewing the regularly worked arable land on the whole surface. In some regions of Sardinia it would have been used, in the past, as soothing for rheumatic pains.[11]. In Sicily it was attributed hemostatic and healing properties. In Tuscany fresh leaves were used to fight the excessive sweating of feet[12]. While in Liguria dried leaves were used by indigent people as tobacco substitute. Used in barns to keep mice away, mixed with hay. The main importance of the classroom lies in the fact that it is a melliferous plant abundantly foraged by bees, especially for the abundant pollen production and long flowering. This plant therefore contributes to the production in late summer and autumn of multi-flower honey and, in areas of strong diffusion, monoflower honey. The Inula viscose is attacked by *Myopites stylatus*, a galligenous Diptera Tefritide. This insect represents the wintering host of *Eupelmus urozonus*, a polyphagous parasitoid of Hymenoptera Chalcidoids, which carries out 2-3 generations per year on the olive fly. Since Eupelmus is the most active natural antagonist of the olive fly, the spread of the inula in the uncultivated areas of olive groves is essential because it can contribute to the control of the

 

phytophagus in integrated pest management programs. The most developed branches of the plant are one of the preferential sites for the nesting of paper wasps belonging to the genus Polistes, in particular Polistes gallicus, a species with a typical Mediterranean distribution.
Studies on costic acid seem to highlight its toxic capabilities against Aphis nerii and varroa [7].

Numerous studies of *I. viscosa* extracts have shown antifungal activity for phytopathogenic fungi and secondary plant metabolites, they are known to have insecticidal, antifungal, acaricidal, antibacterial and cytotoxic activity [14,16]. This test also shows biostimulating effects in terms of vegetative and radical growth of plants and improvement of seeds germination. Very interesting aspects that make *Inula viscosa* a possible alternative to be used as a liquid biostimulant and in the control of insects and pathogenic fungi.

Table 1 - evaluation of *Inula viscosa*, algae and microorganisms on agronomic characters on plants of *Lactuca sativa* L.

| ***Lactuca sativa*** | **PG%** | **FA ($mm^2$)** | **RL (mm)** | **LP (n°)** | **LR (n°)** | **VP (mg)** | **RP (mg)** |
|---|---|---|---|---|---|---|---|
| **CTRL** | 59,79 $^{c}$ | 219,64 $^{c}$ | 80,94 $^{d}$ | 7,40 $^{c}$ | 4,63 $^{d}$ | 832,20 $^{d}$ | 42,96 $^{d}$ |
| **MC** | 67,87 $^{b}$ | 228,42 $^{b}$ | 88,47 $^{c}$ | 11,00 $^{b}$ | 7,22 $^{bc}$ | 837,84 $^{c}$ | 46,84 $^{c}$ |
| **AG** | 68,78 $^{b}$ | 226,40 $^{b}$ | 90,62 $^{b}$ | 11,60 $^{b}$ | 8,20 $^{b}$ | 840,27 $^{b}$ | 49,06 $^{b}$ |
| **INU** | 68,98 $^{b}$ | 228,58 $^{b}$ | 88,98 $^{bc}$ | 12,00 $^{b}$ | 7,05 $^{c}$ | 839,28 $^{bc}$ | 48,87 $^{b}$ |
| **INORT** | 73,13 $^{a}$ | 234,10 $^{a}$ | 94,29 $^{a}$ | 14,00 $^{a}$ | 9,40 $^{a}$ | 843,69 $^{a}$ | 50,72 $^{a}$ |
| **ANOVA** | *** | *** | *** | *** | *** | *** | *** |

One-way ANOVA; n.s. – non significant; *,**,*** – significant at $P \leq 0.05$, 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test ($P = 0.05$).
Parameters: PG% = percentage of germination; FA = foliar area ($mm^2$); RL = root lenght (mm); LP = leaves per plant; LR = lateral roots; VP = Vegetative parts; RP = Radical parts. Treatments: CTRL=control; MC=microorganisms; INU=Inula viscosa; INORT=Inula viscosa+microorganisms+algae; AG= algae

Table 2 - evaluation of *Inula viscosa*, algae and microorganisms on agronomic characters on plants of *Spinacia oleracea* L.

| ***Spinacia oleracea*** | **PG%** | **FA ($mm^2$)** | **RL (mm)** | **LP (n°)** | **LR (n°)** | **VP (mg)** | **RP (mg)** |
|---|---|---|---|---|---|---|---|
| **CTRL** | 64,04 $^{d}$ | 213,18 $^{d}$ | 80,80 $^{c}$ | 6,64 $^{c}$ | 5,04 $^{d}$ | 69,30 $^{d}$ | 30,36 $^{d}$ |
| **MC** | 68,15 $^{c}$ | 218,89 $^{c}$ | 85,36 $^{b}$ | 8,04 $^{b}$ | 6,63 $^{c}$ | 74,34 $^{c}$ | 33,26 $^{c}$ |
| **AG** | 70,27 $^{b}$ | 220,88 $^{b}$ | 86,42 $^{b}$ | 8,63 $^{b}$ | 7,41 $^{b}$ | 76,22 $^{b}$ | 34,88 $^{b}$ |
| **INU** | 69,96 $^{b}$ | 221,68 $^{b}$ | 86,46 $^{b}$ | 8,41 $^{b}$ | 6,42 $^{c}$ | 75,38 $^{bc}$ | 34,21 $^{bc}$ |
| **INORT** | 74,49 $^{a}$ | 227,53 $^{a}$ | 92,77 $^{a}$ | 11,61 $^{a}$ | 9,43 $^{a}$ | 79,34 $^{a}$ | 37,27 $^{a}$ |
| **ANOVA** | *** | *** | *** | *** | *** | *** | *** |

One-way ANOVA; n.s. – non significant; *,**,*** – significant at $P \leq 0.05$, 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test ($P = 0.05$). Parameters: PG% = percentage of germination; FA = foliar area ($mm^2$); RL = root lenght (mm); LP = leaves per plant; LR = lateral roots; VP = Vegetative parts; RP = Radical parts. . Treatments: CTRL=control; MC=microorganisms; INU: Inula viscosa; INORT=Inula viscosa+microorganisms+algae; AG= algae

Table 3 - evaluation of *Inula viscosa*, algae and microorganisms on protection against *Pythium sp.*

| **Groups** | ***Lactuca sativa L.*** ***Pythium sp.*** **plants affected (n°)** | ***Spinacia oleracea L.*** ***Pythium sp.*** **plants affected (n°)** |
|---|---|---|
| **CTRL** | 3,44 $^{a}$ | 3,81 $^{a}$ |
| **MC** | 1,61 $^{b}$ | 2,28 $^{b}$ |
| **AG** | 3,05 $^{a}$ | 3,43 $^{a}$ |
| **INU** | 1,83 $^{b}$ | 3,46 $^{a}$ |
| **INORT** | 0,82 $^{c}$ | 1,20 $^{c}$ |
| **ANOVA** | *** | ** |

One-way ANOVA; n.s. – non significant; *,**,*** – significant at $P \leq 0.05$, 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test ($P = 0.05$) Treatments: CTRL=control; MC=microorganisms; INU=Inula viscosa; INORT=Inula viscosa+microorganisms+algae; AG= algae

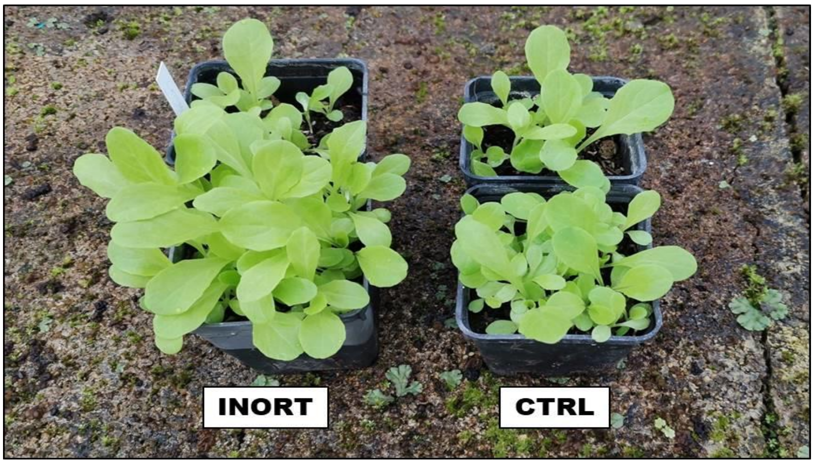


Figure 3 - Effect of INORT on vegetative biomass of *Lactuca sativa L.*
Legend: (CTRL): control; (INORT): Inula+microorganisms+algae

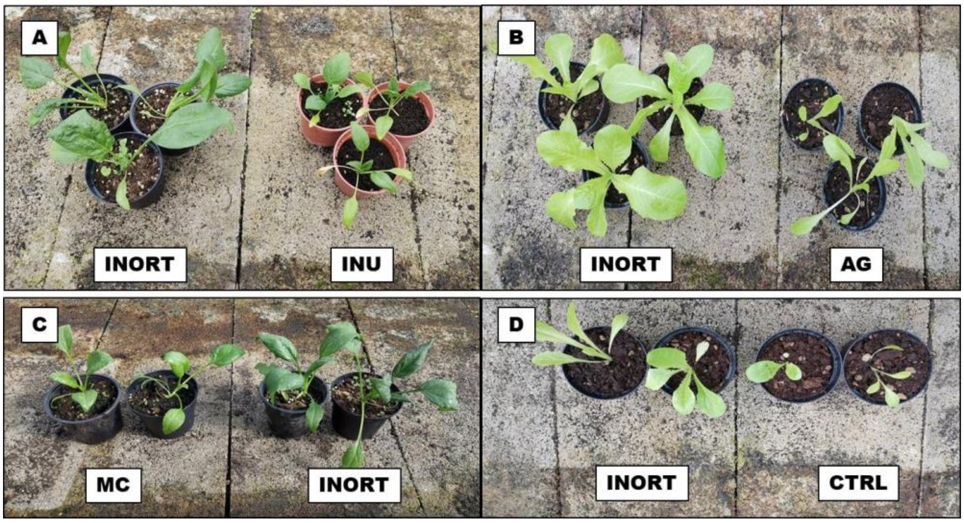


Figure 4 – Effect of different plant biostimulant on vegetative growth of *Spinacia oleracea* (Inort vs Ctrl) (A), *Lactuca sativa* (INORT vs AG) (B), *Spinacia oleracea* (MC vs INORT) (C), *Lactuca sativa* (INORT vs CTRL) (D).
Legend: (CTRL) control; (MC): microorganisms; (AG): algae; (INORT): microorganisms+algae+inula

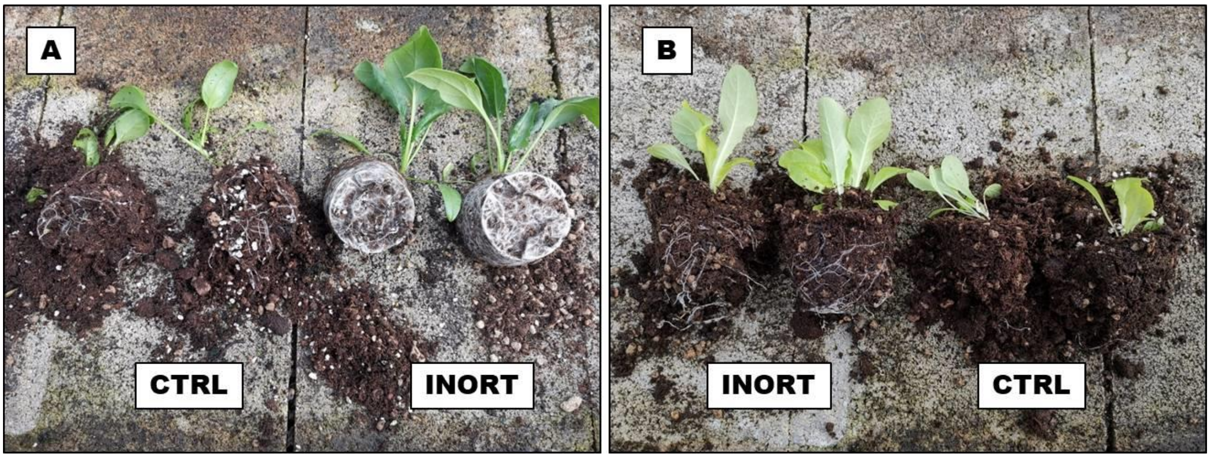


Figure 5 – Effect of INORT on roots growth of *Spinacia oleracea* (A) and *Lactuca sativa* (B)
Legend: (CTRL) control; (INORT): microorganisms+algae+inula

## V. CONCLUSION

The test has shown that the use of a liquid biostimulant based on Inula viscose can significantly improve seed germination, plant growth and the quality of *Lactuca sativa* L. and *Spinacia oleracea* L. The continued application of this biostimulant has also indirectly improved the plant's defenses against *Pythium sp.* by significantly reducing seedling mortality.

Therefore, further interesting and innovative aspects of the use of this plant have been highlighted, given its acknowledged importance from a medicinal, melliferous and biodiversity point of view. Further experiments are currently underway to confirm the use of INORT as a possible product that can guarantee the grower a possible reduction of fertilizers and plant protection in horticulture.

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