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Improving Quality and Production of Horticultural Crops Through the Use of A Biostimulant Based on *Inula Viscosa* and Control of Seedling Pathogens

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Abstract- In this research work the possibility of using a biostimulant based on *Inula viscosa* L. *(Dittrichia viscosa* L.*)* to improve the growth and quality of *Licopersicon esculentum L., Daucus carota, Pak choi, Perilla frutescens Petroselinum crispum* plants. was evaluated. Additionally, in the experiment the presence of plant mortality following attacks of *Pithyum spp.*and *Fusarium sp.* has been examined. 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 viscosa, irrigated with water and substrate previously fertilized; v) group with (INORT: mix Microorganisms + Inula viscosa + Algae), irrigated with water and substrate previously fertilized. The trial showed that all *Lycopersicon esculentum L, Daucus carota*, Pak choi, *Perilla frutescens* and *Petroselinum crispum* plants treated with Inula viscosa biostimulant showed 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 plant protection from *Pithyum spp.* and *Fusarium sp.*

Further researches will be carried out on the use of Inula as biostimulant in order to investigate if there is a specificity of improvement or worsening according to the type of plant on which it is used, if there are differences according to the type of extraction, methods and times of plant harvesting, association or not with other biostimulants of natural origin. The use of Inula as a biostimulant associated with algae and microorganisms in this and other experiments has shown significant effects in the cultivation of plants that, although deserving further investigation, are certainly of interest for farmers.

Keywords-Sustainable applications; Plant quality; Vegetables; Plant growth promoting rhizobacteria; Biocontrol

I. INTRODUCTION

Since the past times it has always been of great importance the utilisation of wild plants as a resource of nutritive substances and medicinal principles; subsequently the preparations based on plants have been substituted by synthetic products, even though today they are getting more and more important. *Inula viscosa* (Figure 1) belongs to Asteraceae (Compositae), it represents an important family of herbaceous, shrubby and sometimes arboreal plants characterized by the presence of particular flower heads. They are spread all over the world even though their ideal climate is that of subtropical and temperate regions [1,2].

The flowers are characterized by a modified disc-shaped receptacle, differentiated into tubular (usually in the center). Generally pollinated by bees, they produce fruits called achenes, which may or may not have a pappus (bristle-like appendage), which allows dispersal by wind.

Inula is defined as a plant of Mediterranean regions, present in Southern Europe, North Africa, it is easily recognizable in rural environments and by the side of the road. Prefers calcareous and clayey soil, often acid and it can be found between 0 and 800 m above sea level. Because of its diffusion, it is considered as a weed and since many years there have been many scientific studies which have revaluated its potentialities [3,4].

Inula is an important plant for caterpillars of butterflies and moths, characterized by a high melliferous power, it has a very long flowering period from late spring to early autumn, therefore it represents an important source of nutrition for insects [5,6].

II. RELATED WORK

Inula viscosa is now a much studied plant from the agronomic point of view (the components of the extracts are evaluated as antigerminants, desiccants and for the biological control of insects and fungi) or for the preparation of medicines. Easily cultivable, with a long growth phase, it is adaptable to hostile and arid environments [7,8].

Numerous studies have been carried out to isolate useful active compounds from the plant, such as mono-, sesqui- and tri-terpenes, flavones, flavanones and carbohydrates. Some identified metabolites have demonstrated antifungal capabilities, antioxidant properties, and anti-inflammatory effects [9,10]. In the agricultural field numerous researches have demonstrated the ability to control different pathogens and in some cases to stimulate the growth of vegetable and ornamental plants [11,12,13].

Studies in general have shown Inula:
- Shows in some cases allelopathic activity against some plant species [14,4];
- represents a refuge zone for useful insects for biological control of plant pests [15];
- can be used as a phytodepurative plant in soils contaminated by heavy metals [16,17];
- it has a biostimulant action towards many plant species, this depends on the way and the type of production of the extract, the growth conditions of the plant, the protocol and timing of use [3,12];
- applied in the form of gel to be vaporized, it has shown important effects in the control of varroa and nosema of bees, through mechanisms of olfactory disorientation and sterilization of the environments frequented by bees [18,19,20].

In this research work the possibility of using a biostimulant based on *Inula viscosa* L. *(Dittrichia viscosa* L.*)* (Figure 1) to improve the growth and quality of *Licopersicon esculentum L., Daucus carota, Pak choi, Perilla frutescens Petroselinum crispum* (Figure 2) plants. 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 *Licopersicon esculentum L., Daucus carota, Pak choi, Perilla frutescens Petroselinum crispum* plants

Plants were initially sown in 12 cm pots to evaluate the germination percentage and the average germination time, then they were transplanted in 16 and 18 cm pots to evaluate the growth after the different treatments; for the cultivation test 30 rooted cuttings per thesis, divided into 3 replicas of 10 plants each were used. 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 5 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 January 14, 2021, percentage of seed emergence, plant height, number of leaves per plant, aerial part and root system (g), were recorded. For *Lycopersicon esculentum L.* , plant height at 70 days after planting , plant nodes at 70 days after planting, leaf area index, total dry biomass, fruit fresh weight and total fruit number have been evaluated

Additionally, in the experiment the presence of plant mortality following attacks of *Pithyum spp.*and *Fusarium sp.* has been examined.

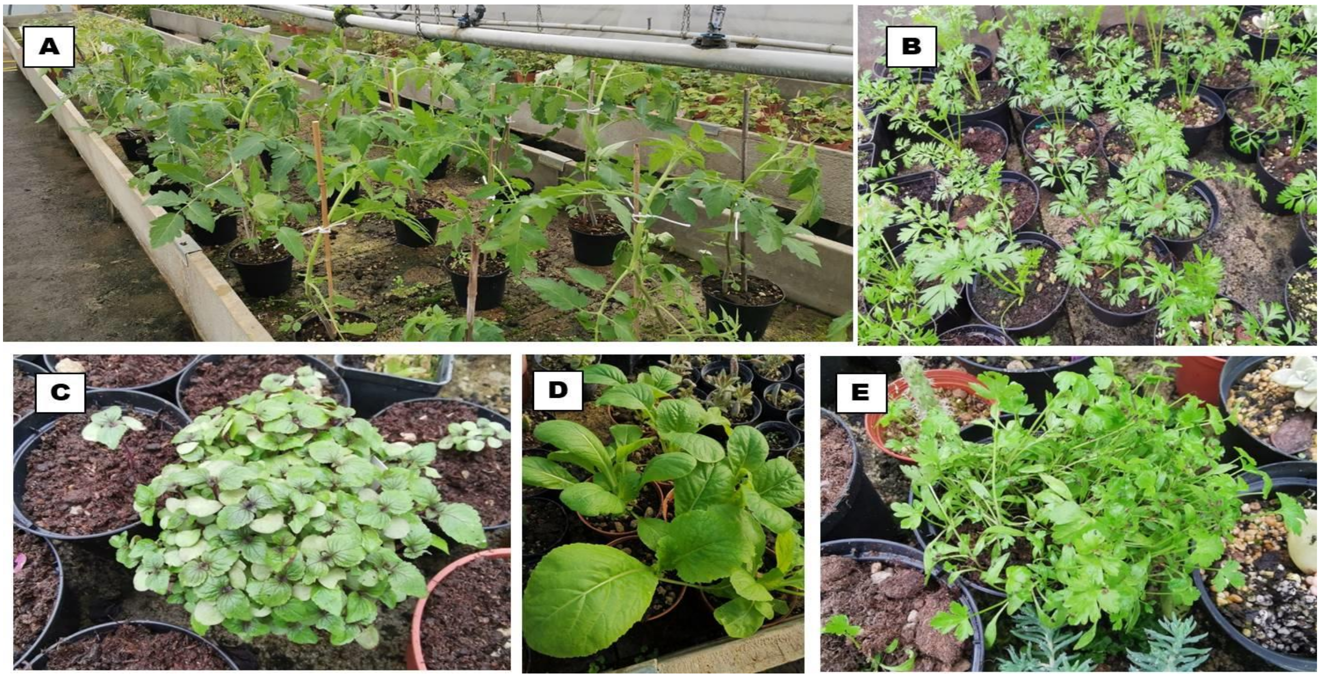

Figure 2 – Details of *Licopersicon esculentum (A), Daucus carota (B), Perilla frutescens (C), Pak choi (D) and Petroselinum crispum (E) in greenhouse*

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

The trial showed that all *Lycopersicon esculentum L*, *Daucus carota*, Pak choi, *Perilla frutescens* and *Petroselinum crispum* plants treated with Inula viscosa biostimulant showed significant increase in the agronomic parameters analyzed in comparison to control (CTRL). The test data show that the application 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 plant protection from *Pithyum spp.* and *Fusarium sp.*

In *Lycopersicon esculentum L.* (Table 1), treatment with (INORT) significantly improved plant $height_{70}$ with 138,70 cm, while the untreated control was the worst thesis with 125.62 cm (Figure 3). With regard to number of $nodes_{70}$ per plant (INORT) was the best thesis with 30.80, followed by (AG) with 28.00, (INU) with 27.60, (MC) with 26.20 and (CTRL) with 22.80. Concerning LAI_{tot} (INORT) was the top thesis with 3.04 m2m-2, closely followed by (INU) and (AG) with 2.86 m^2m^{-2} and 2.80 m^2m^{-2} respectively, (MC) 2.47 m^2m^{-2} and the untreated control with 2.27 m^2m^{-2}. For plant dry weight (DW), the thesis (INORT) with 446.50 g m-2, as well as for fruit fresh weight with 9.96 kg m-2 and fruit number 77.80. The thesis (INORT) was also the best for biocontrol of *Pythium spp.* with 0.23, while there were no significant differences in the control of *Fusarium sp.* between the different theses.

In *Daucus carota*, (Table 2) the seed germination rate was significantly higher in (INORT) with 85%, followed by (INU) 82%, (AG) 80%, (MC) 77% and finally the untreated control with 74%. Regarding plant height (PH), the thesis that showed better results was (INORT) with 35,80 cm, followed by (AG) and (INU) with 34,45 cm and 34,28 cm respectively , (MC) with 31,67 cm and lastly (CTRL) with 24,82 cm. The INORT treatment was also the best in terms of leaves number, vegetative weight and roots weight (Figure 4). All treatments showed a positive control against *Pythium spp.* and *Fusarium spp.*, in particular the INORT treatment seems to be the one that reduced most the mortality of plants.

In Pak choi, (Table 3) the seed germination rate was significantly higher in (INORT) with 86%, followed by (INU) 70%, (AG) 66%, (MC) 65% and finally the untreated control with 60%. Regarding plant height (PH), the thesis that showed better results was (INORT) with 38.73 cm, followed by (AG) and (INU) with 37.96 cm and 37.86 cm respectively, (MC) with 36.98 cm and finally (CTRL) with 34.49 cm (Figure 5). The INORT treatment was also the best in terms of number of leaves, vegetative weight, and root weight. All treatments showed positive control against *Pythium spp.* while INORT and INU were the best for *Fusarium sp.*

In *Perilla frutescens*, (Table 4) the seed germination rate was significantly higher in (INORT) with 90%, followed by (INU) 86%, (AG) 85%, (MC) 85% and finally the untreated control with 80%. Regarding plant height (PH), the thesis that showed better results was (INORT) with 15.40 cm, followed by (INU) and (AG) with 14.74 cm and 14.67 cm respectively, (MC) with 13.69 cm and finally (CTRL) with 12.16 cm. The INORT treatment also on this plant species was the best in terms of number of leaves, vegetative weight, and root weight (Figure 6A). No treatment showed biocontrol effects against *Pythium spp.* while all treatments showed a positive control on *Fusarium sp.* compared to the untreated control.

In *Petroselinum crispum*, (Table 5) the seed germination rate was significantly higher in (INORT) with 90%, followed by (INU) 78%, (AG) 74%, (MC) 72% and finally the untreated control with 68%. Regarding plant height (PH), the thesis that showed better results was (INORT) with 38.03 cm, followed by (AG) and (INU) with 37.54 cm and 37.25 cm respectively, (MC) with 36.65 cm and finally (CTRL) with 35.89 cm (Figure 6B). The INORT treatment showed improvement in leaf number, vegetative weight, and root weight. All treatments showed improvement in *Pithyum spp.* control, while only (INORT), (INU), and (MC) showed significant effects against *Fusarium sp.*

The results of this experiment have highlighted the possible use of a biostimulant based on Inula viscosa in improving the quality of vegetable plants such as *Lycopersicon esculentum L.*, *Daucus carota*, Pak choi, *Perilla frutescens* and *Petroselinum crispum*. In literature there are some researches showing that the use of Inula can inhibit in some way the germination and development of some plants. This effect is probably due to the metabolite of Inula used in the experiments, to the method and time of preparation of the extract and to the phase of growth and the type of tissue of the plant from which metabolites are taken. In this research as in others previously carried out on other horticultural and ornamental plants, the use of Inula macerate associated or not with other compounds of natural origin such as algae or microbial-based biofertilizers has shown a significant improvement in the growth of plants under experimentation. Inula has also shown in several cases a possible effect in the control of some seedling pathogens such as *Pithyum spp.* and *Fusarium sp* [3,12].

This plant as well as having an important role in agriculture, used in the form of biostimulant or repellent extract in the control of some insect pathogens, assumes a fundamental role for plant biodiversity, because on it find a possible refuge antagonists of the olive fly as *Eupelmus urozonus*, a polyphagous parasitoid of Hymenoptera Chalcidoids [15]. Further studies have been carried out to evaluate the inula in the control of varroa in bees, both by extracting the costic acid and by using it inside a gel to be vaporized which has given numerous positive results [18,19,20]. Numerous studies of I. viscosa extracts have shown antifungal activity for phytopathogenic fungi and secondary plant metabolites, are recognized to have an insecticide, antimycotic, acaricidal, antimicrobial and phytotoxic activity [21,6,15].

Further research will need to be done to improve protocols of use and standardize methods of sampling and extraction and to expand the number of plants on which inula is used as a possible biostimulant.

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

Licopersicon esculentum L.	**PH_{70} (cm/plant)**	**PN_{70} (n°/plant)**	**LAI tot (m^2m^{-2})**	**DWtot ($g\ m^{-2}$)**	**FW ($Kg\ m^{-2}$)**	**FN (n°)**	**PA (n°)**	**PF (n°)**
CTRL	125,62 d	22,80 d	2,27 d	427,51 c	8,57 d	60,20 d	3,00 a	1,40 a
MC	132,28 c	26,20 c	2,47 c	438,56 b	8,86 c	64,40 c	1,20 b	1,20 ab
AG	133,62 bc	28,00 b	2,80 b	445,82 a	8,93 c	71,60 b	1,20 b	1,00 abc
INU	135,44 b	27,60 b	2,86 b	439,77 b	9,14 b	73,00 b	0,80 bc	0,60 bc
INORT	138,71 a	30,80 a	3,04 a	446,50 a	9,96 a	77,80 a	0,23 c	0,40 c
ANOVA	***	***	***	***	***	***	***	**ns**

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: Plant height (PH70) and number of nodes (PN70) measured at 70 days after transplanting; (LAI Tot) total plant defoliations; (DW Tot) plant dry weight; (FW) fruits fresh weight; (FN) fruits number; (PA) number of plants affected by *Pythium spp.;* (PF) number of plants affected by *Fusarium sp*.
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 *Daucus carota*

Daucus carota	**PG%**	**PH (cm)**	**NL (n°)**	**VW (g)**	**RW (g)**	**PA (n°)**	**PF (n°)**
CTRL	74	24,82 d	3,65 c	42,89 d	28,40 e	1,82 a	1,94 a
MC	77	31,67 c	4,81 ab	45,45 c	29,30 d	0,86 b	0,44 b
AG	80	34,45 b	4,82 ab	46,77 b	30,37 c	0,61 b	0,78 b
INU	82	34,28 b	4,63 b	46,79 b	31,27 b	0,45 b	0,65 b
INORT	85	35,80 a	5,40 a	49,09 a	32,78 a	0,22 b	0,41 b
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; PH = plant height (cm); NL = leaves number (n°); VW = vegetative weight; RW = roots weight; (PA) number of plants affected by *Pythium spp.;* (PF) number of plants affected by *Fusarium sp.*
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 agronomic characters on plants of *Pak choi*

Pak choi	PG%	PH (cm)	NL (n°)	VW (g)	RW (g)	PA (n°)	PF (n°)
CTRL	60	34,49 d	5,24 c	54,49 d	34,91 e	2,21 a	1,2 a
MC	65	36,98 c	6,24 b	58,25 c	35,97 d	0,64 b	0,65 ab
AG	66	37,96 b	5,84 bc	60,99 b	36,62 c	1,00 b	0,84 ab
INU	70	37,86 b	5,61 bc	60,77 b	37,95 b	0,61 b	0,41 b
INORT	76	38,73 a	7,21 a	62,39 a	39,72 a	0,43 b	0,24 b
ANOVA	-	***	***	***	***	**	ns

One-way ANOVA; n.s. – non significant; *,**,*** – significant at P ≤ 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; PH = plant height (cm); NL = leaves number (n°); VW = vegetative weight; RW = roots weight;(PA) number of plants affected by *Pythium spp.;* (PF) number of plants affected by *Fusarium sp.*
Treatments: CTRL=control; MC=microorganisms; INU=Inula viscosa; INORT=Inula viscosa+microorganisms+algae; AG= algae

Table 4 - evaluation of *Inula viscosa*, algae and microorganisms on agronomic characters on plants of *Perilla frutescens*

Perilla frutescens	PG%	PH (cm)	NL (n°)	VW (g)	RW (g)	PA (n°)	PF (n°)
CTRL	80	12,16 d	3,23 c	14,91 d	11,04 d	0,41 a	0,63 a
MC	85	13,69 c	4,24 b	16,03 c	12,63 c	0,00 a	0,00 b
AG	85	14,67 b	4,64 b	16,51 b	13,32 b	0,42 a	0,45 ab
INU	86	14,74 b	4,61 b	16,90 b	13,41 b	0,00 a	0,00 b
INORT	90	15,40 a	5,41 a	17,64 a	14,63 a	0,00 a	0,00 b
ANOVA	-	***	***	***	***	ns	*

One-way ANOVA; n.s. – non significant; *,**,*** – significant at P ≤ 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; PH = plant height (cm); NL = leaves number (n°); VW = vegetative weight; RW = roots weight. (FN) fruits number; (PA) number of plants affected by *Pythium spp.;* (PF) number of plants affected by *Fusarium sp.*
Treatments: CTRL=control; MC=microorganisms; INU=Inula viscosa; INORT=Inula viscosa+microorganisms+algae; AG= algae

Table 5 - evaluation of *Inula viscosa*, algae and microorganisms on agronomic characters on plants of *Petroselinum crispum*

Petroselinum crispum	PG%	PH (cm)	NL (n°)	VW (g)	RW (g)	PA (n°)	PF (n°)
CTRL	68	35,89 d	56,44 d	44,96 d	35,58 d	1,21 a	1,46 a
MC	72	36,65 c	64,00 c	46,67 c	38,06 c	0,00 b	0,24 b
AG	74	37,54 b	66,21 c	47,64 b	40,34 b	0,44 b	0,83 ab
INU	78	37,25 b	71,84 b	48,02 b	40,76 b	0,00 b	0,21 b
INORT	90	38,03 a	77,61 a	49,14 a	42,59 a	0,00 b	0,20 b
ANOVA	-	***	***	***	***	***	**

One-way ANOVA; n.s. – non significant; *,**,*** – significant at P ≤ 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; PH = plant height (cm); NL = leaves number (n°); VW = vegetative weight; RW = roots weight. (PA) number of plants affected by *Pythium spp.;* (PF) number of plants affected by *Fusarium sp.*
Treatments: CTRL=control; MC=microorganisms; INU=Inula viscosa; INORT=Inula viscosa+microorganisms+algae; AG= algae

Figure 3 - Effect of INORT on vegetative biomass of *Licopersicon esculentum L.*
Legend: (CTRL): control; (INORT): Inula+micorganisms+algae

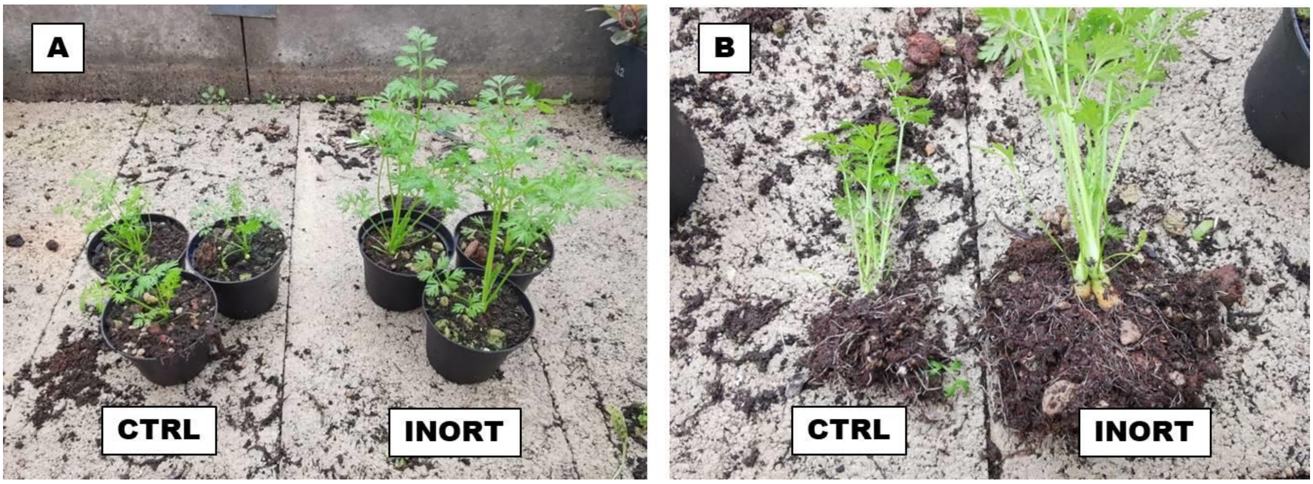

Figure 4 – Effect of INORT on vegetative (A) and roots (B) growth of *Daucus carota*
Legend: (CTRL) control; (INORT): microorganisms+algae+inula

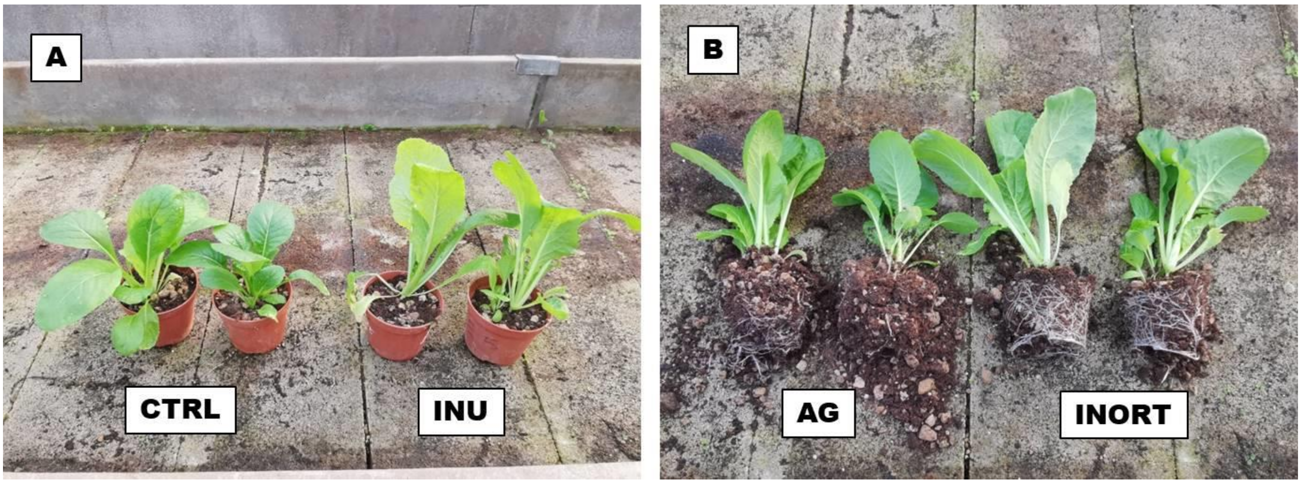

Figure 5 – Effect of INU (A), Inort (B) and AG (B) on vegetative and roots biomass of Pak choi
Legend: (CTRL) control; (INU): Inula; (AG): algae; (INORT): microorganisms+algae+inula

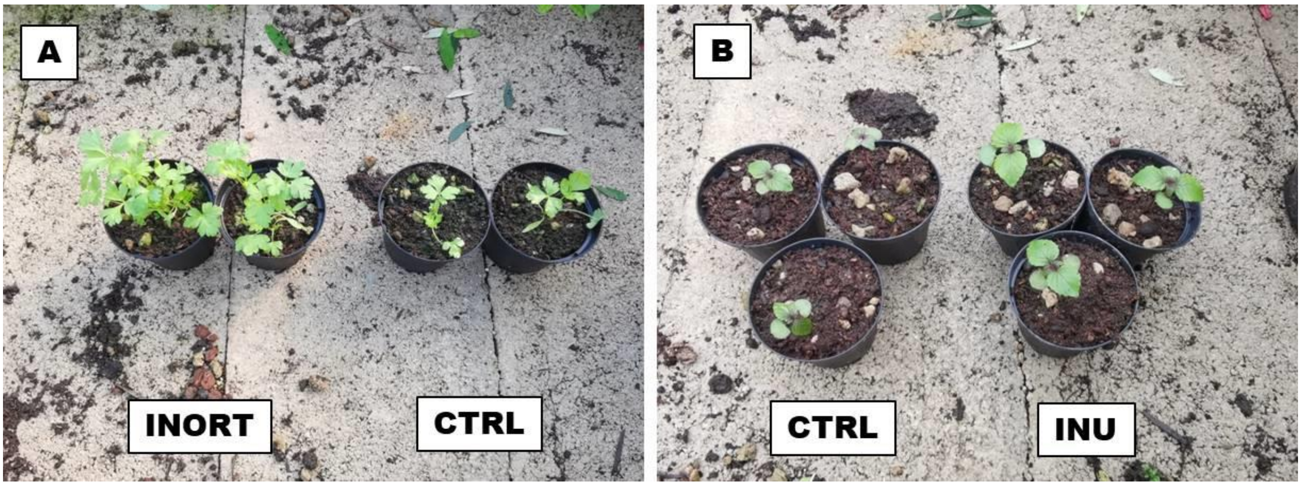

Figure 6 – Effect of INORT and INU on vegetative growth of *Petroselinum crispum* (A) and *Perilla frutescens* (B)
Legend: (CTRL) control; (INORT): microorganisms+algae+inula; (INU): Inula

V. CONCLUSION

This experiment showed that the use of a liquid biostimulant based on Inula viscosa can significantly improve the quality and production of of *Lycopersicon esculentum L.*, *Daucus carota*, Pak choi, *Perilla frutescens* and *Petroselinum crispum* plants. Positive effects were also found regarding the control of possible seedbed pathogens such as Pythium spp. and Fusarium sp.

Further researches will be carried out on the use of Inula as biostimulant in order to investigate if there is a specificity of improvement or worsening according to the type of plant on which it is used, if there are differences according to the type of extraction, methods and times of plant harvesting, association or not with other biostimulants of natural origin. The use of Inula as a biostimulant associated with algae and microorganisms in this and other experiments has shown significant effects in the cultivation of plants that, although deserving further investigation, are certainly of interest for farmers.

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