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INORT: Biofertilizer based on *Inula viscosa L.* (*Dittrichia viscosa L.*), algae and micro-organisms for growth, *Fusarium oxysporum* defence and water stress resistance of *Plumeria frangipani*

Domenico Prisa [1, *] and Francesco Attanasio [2]

[1] CREA Research Centre for Vegetable and Ornamental Crops, Council for Agricultural Research and Economics, Via dei Fiori 8, 51012 Pescia, PT, Italy.
[2] Attanasio Farm, Via Matteotti 35, San Sebastiano al Vesuvio, NA, Italy.

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Abstract

Research objective: In this study, the possibility of using INORT, a biostimulant based on *Inula viscosa* L. (*Dittrichia viscosa* L.), algae and microorganisms to improve growth, defence against *Fusarium oxysporum* and resistance to water stress, on *Plumeria frangipani* plants grown in open field was evaluated.

Materials and Methods: The experiments, started in January 2023, were conducted in the greenhouses of CREA-OF in Pescia (Pt), on Plumeria frangipani plants cvs "California sunset and "Super round". The plants were grown in open field. The five experimental groups in cultivation were: group without biostimulant (CTRL); group with microrganisms; group with algae; group with *Inula viscosa*; group with (INORT) (mix Microorganisms + Inula viscosa + Algae).The product INORT was supplied by the Francesco Attanasio farm. On 15 September 2023, plant height, number of leaves, number of branches, total leaf area per plant (mm^2), primary root length (mm), aerial and root system biomass were recorded. In addition, plant mortality as a result of Fusarium oxysporum attacks and water stress was assessed in the experiment.

Results and Discussion: The experiment showed that the use of the biostimulant based on Inula viscosa, microorganisms and algae (INORT) can indeed significantly improve the vegetative and root growth of *Plumeria frangipani* plants grown in the open field. Improvements were also found in plant height, number of leaves, number of floral branches, leaf area, vegetative and root biomass and root length at different irrigation frequencies of 3 and 6 days. A very interesting aspect was also the ability of the biostimulant based on *Inula viscosa*, microorganisms and algae to significantly reduce the incidence of *Fusarium oxysporum* attacks on leaves.

Conclusions: Given the importance of *Inula viscosa* from a medicinal, pollination and biodiversity point of view, new agricultural experiments are very important as they could allow the development of new biofertiliser products that can be used in organic and sustainable farming systems

Keywords: Microorganisms; Sustainable applications; Plant extract; Rhizosphere; Plant stimulation

1. Introduction

A perennial bushy plant (*Inula viscosa* L., also known as *Dittrichia viscosa* Greuter) belonging to the family *Compositae*, it is considered the largest among Phanerogams and includes about 959 genera, which are commonly found in Mediterranean areas [1]. An erect cauli, woody at the base, richly covered with leaves, and a height of 50 to 150 cm, I. viscosa has a characteristic smell, with erect cauli, woody at their base and thickly covered in leaves. Inflorescences

* Corresponding author: Domenico Prisa

consist of numerous pyramidal flower heads with golden yellow flowers with linear-lanceolate leaves. In autumn, flowers bloom, and fruits are made up of achenes. Many studies have been conducted in relation to the presence of numerous compounds of different chemical nature in different parts of the plant (roots, stems, leaves, flowers, etc..), whose properties make some potential applications in pharmaceuticals, cosmetics, aromatics, and related fields [2]. Folk medicine uses this species primarily to treat liver disorders, including analgesic, anti-inflammatory, antipyretic, anthelmintic, and antifungal properties [3,4]. Although it is described as a typical plant of the Mediterranean region [5,6,7], it is actually found in southern Europe and North Africa, but no precise habitats of growth have been identified. It is very easy to recognize in rural environments and near roadsides, prefers calcareous and clayey soil, often acidic and being a heliophilous species, it can be found at a range of elevations from 0 to 800 m.a.s.l. For several years no scientific studies were conducted on its potential, but recently it has been revalued [8]. As a plant abundantly foraged by bees, I. viscosa provides a source of nourishment for butterfly caterpillars and moths [9,10]. Furthermore, it is recognized for its high melliferous power. Inulas bloom very long between late spring and early autumn, when most plants are generally no longer in bloom. As a result, the classroom has a high potential for producing swarms in the following spring, as well as honey in the autumn and late summer [11,12].

2. Related Work

Several studies have reported that I. viscose extracts have the ability to control pathogenic fungi, while secondary plant metabolites have been shown to be insecticidal and antibacterial [14,16]. As a result of several studies it has been demonstrated that Inula 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 INORT, a biostimulant based on *Inula viscosa* L. (*Dittrichia viscosa* L.), algae and microorganisms to improve growth, defence against *Fusarium oxysporum* and resistance to water stress, on *Plumeria frangipani* plants grown in open field was evaluated.

Figure 1 Details of the *Plumeria frangipani* plants used in the trial

3. Materials and methods

The experiments, started in January 2023, were conducted in the greenhouses of CREA-OF in Pescia (PT), on *Plumeria frangipani* plants cvs "California sunset and "Super round" (Figure 1).

The plants were grown in open field; 30 rooted cuttings per thesis, divided into 3 replications of 10 plants each planted in early January 2023. All plants were fertilised with a slow-release fertiliser (2 kg m^{-3} of 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 once every 3 days an 6 days (two treatments with different irrigation intervals for each thesis to assess any difference in water stress resistance) and cultivated for 9 months. The plants were drip-irrigated. Irrigation was activated by a timer whose schedule was adjusted weekly according to the weather conditions and the leaching fraction. On 15 September 2023, plant height, number of leaves, number of branches, total leaf area per plant (mm^2), primary root length (mm), aerial and root system biomass were recorded. In addition, plant mortality as a result of *Fusarium oxysporum* attacks and water stress was assessed in the experiment.

3.1. 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).

4. Results and Discussion

The experiment showed that the use of the biostimulant based on *Inula viscosa*, microorganisms and algae (INORT) can indeed significantly improve the vegetative and root growth of *Plumeria frangipani* plants grown in the open field (Table 1 and Table 2). All treatments showed a significant improvement over the untreated control for the agronomic parameters analysed, but the INORT treatment was statistically the best (Figure 2). Improvements were also found in plant height, number of leaves, number of floral branches, leaf area, vegetative and root biomass and root length at different irrigation frequencies of 3 and 6 days (Figure 3). A very interesting aspect was also the ability of the biostimulant based on *Inula viscosa*, microorganisms and algae to significantly reduce the incidence of *Fusarium oxysporum* attacks on leaves.

Typically heliophilous and ruderal, Inula viscosa is commonly found in uncultivated areas, ruins, along roads, headlands, cliffs and escarpments. Its ruggedness and adaptability allow it to colonise poor, dry and stony soils [24,25]. It has sticky leaves and an odour that repels livestock. When invasive, it tends to grow in degraded pastures and in the rows of extensive plantations (vineyards, olive groves, orchards), but avoids arable land that is regularly cultivated. The plant was used in some areas of Sardinia as a remedy for rheumatic pains [26]. In Sicily it was used for healing and haemostatic purposes. In Tuscany, fresh leaves were used against excessive sweating. In Liguria, dried leaves were used by the poor as a substitute for tobacco. In barns it was mixed with hay to repel mice. As a melliferous plant, it is highly favoured by bees, mainly because of its abundance of pollen and its long flowering period. As a result, this plant produces multifloral honey in late summer and autumn, and monofloral honey in areas of high abundance. Inulas are attacked by a tephritid gall dipteran called *Myopites stylatus* [27,28]. The olive fly is the overwintering host of *Eupelmus urozonus*, a polyphagous parasitoid of *Hymenoptera calcidoides* that reproduces 2-3 times per year [29,30]. Integrated pest management programmes can contribute to the control of the phytophage by spreading *Eupelmus,* inula in uncultivated areas of olive groves, which is essential as *Eupelmus* is the most active natural antagonist of the olive fly [31,32]. In particular, *Polistes gallicus*, a species with a typical Mediterranean distribution, nests on the most developed branches of the plant [33,34]. *Aphis nerii* and Varroa appear to be susceptible to the toxic effects of costic acid. The antifungal activity of *I. viscosa* extracts against phytopathogenic fungi and secondary metabolites has been demonstrated in numerous studies, as well as its insecticidal, antifungal, acaricidal, antibacterial and cytotoxic properties [35]. In addition, biofertilising activity has been demonstrated in combination with microorganisms and algae on a variety of vegetable species [36]. Biostimulative effects have also been demonstrated in field-grown plants, where plants are more resistant to water stress and to pathogenic fungi such as *Fusarium oxysporum*. *Inula viscosa* has a number of interesting properties that make it a potential liquid biostimulant and an effective means of controlling pathogenic insects and fungi.

Table 1 Evaluation of *Inula viscosa,* algae and microorganisms on agronomic characters on plants of *Plumeria frangipani* cv California Sunset

California Sunset	Rounds (days)	PH (cm)	LN (n°)	NB (n°)	TLA (mm^2)	VW (g)	RW (g)	RL (cm)	FO attack (n°)
CTRL	3	52.57 d	31.22 c	1.41 c	122.24 d	76.46 d	51.94 d	3.65 e	5.61 a
MC	3	59.38 b	34.23 b	2.22 b	124.99 c	78.18 c	53.84 c	4.13 d	3.63 b
AG	3	59.39 b	34.81 b	2.42 b	126.10 b	79.02 b	54.61 b	5.09 b	3.82 b
INU	3	55.82 c	34.82 b	2.43 b	124.75 c	77.96 c	53.73 c	4.81 c	2.84 c
INORT	3	61.74 a	38.00 a	3.61 a	132.17 a	80.52 a	56.61 a	5.47 a	1.00 d
ANOVA	-	***	***	***	***	***	***	***	***
CTRL	6	42.76 d	21.81 d	1.20 b	75.10 d	48.36 e	35.31 d	3.12 e	6.82 a
MC	6	48.28 b	25.00 b	2.00 a	79.40 b	50.48 c	38.57 b	4.35 b	3.83 b
AG	6	48.44 b	24.80 b	2.22 a	79.51 b	51.40 b	38.68 b	4.17 c	3.64 b
INU	6	45.77 c	23.00 c	1.41 b	77.84 c	49.37 d	37.57 c	3.76 d	4.22 b
INORT	6	49.55 a	27.61 a	2.22 a	83.93 a	52.27 a	49.39 a	5.02 a	2.21 c
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: PH = plant height (cm); LN = leaves number (cm); NB = branches number (n°); TLA = total leaves area (mm^2); VW = vegetative weight (g); RW = roots weight (g); RL = roots length (cm); FO= plants affected by *Fusarium oxysporum* (n°). 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 *Plumeria frangipani* cv Super Round

Super Round	Rounds (days)	PH (cm)	LN (n°)	NB (n°)	TLA (mm^2)	VW (g)	RW (g)	RL (cm)	FO attack (n°)
CTRL	3	52.59 d	32.41 c	1.82 c	121.89 d	76.48 d	51.97 d	3.69 e	6.00 a
MC	3	59.39 b	34.82 b	2.42 bc	125.21 c	78.27 c	53.86 c	4.28 d	3.82 b
AG	3	59.41 b	35.00 b	2.61 b	126.14 b	79.06 b	56.64 b	5.12 b	3.43 b
INU	3	55.82 c	35.00 b	2.61 b	124.76 c	78.16 c	53.74 c	4.82 c	2.61 c
INORT	3	61.75 a	38.21 a	3.82 a	132.77 a	80.63 a	56.84 a	5.48 a	0.64 d
ANOVA	-	***	***	***	***	***	***	***	***
CTRL	6	42.77 d	22.22 d	1.42 c	75.31 d	48.39 e	35.32 d	3.17 d	7.42 a
MC	6	48.29 b	25.42 b	2.21 ab	79.58 b	50.56 c	38.66 b	4.37 b	3.61 b
AG	6	48.45 b	25.00 b	2.63 a	79.56 b	51.51 b	38.73 b	4.26 b	3.23 b
INU	6	45.78 c	23.41 c	1.63 bc	77.85 c	49.46 d	37.59 c	3.77 c	3.42 b
INORT	6	49.56 a	28.41 a	2.82 a	84.08 a	52.43 a	43.66 a	5.11 a	1.64 c

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: PH = plant height (cm); LN = leaves number (cm); NB = branches number (n°); TLA = total leaves area (mm^2); VW = vegetative weight (g); RW = roots weight (g); RL = roots length (cm); FO= plants affected by *Fusarium oxysporum* (n°). Treatments: CTRL=control; MC=microorganisms; INU=Inula viscosa; INORT=Inula viscosa+microorganisms+algae; AG= algae

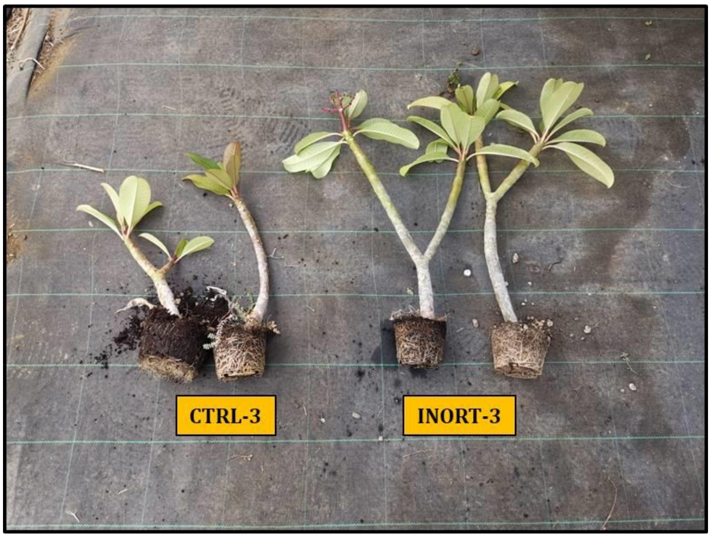

Figure 2 Effect of INORT on vegetative biomass, plant height, number of branches of *Plumeria frangipani* cv "California Sunset". Turnover 3-day water regime. Legend: (CTRL): control; (INORT):Inula+microorganisms+algae

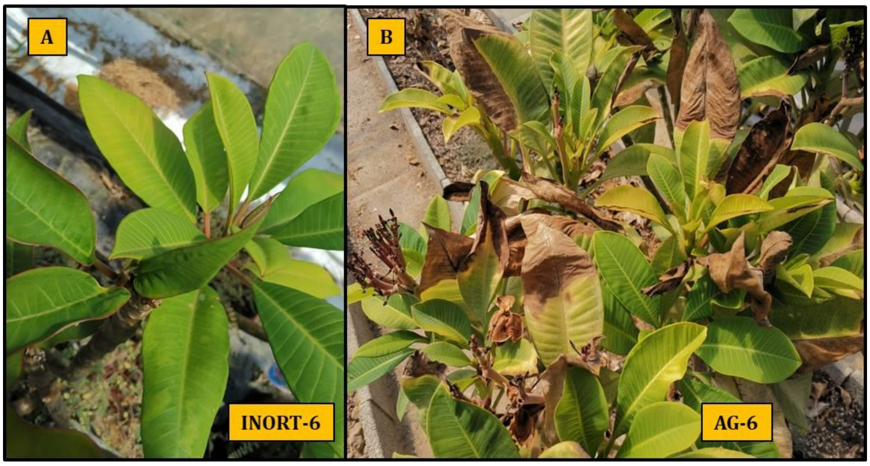

Figure 3 Effect on leaf resistance to water stress of treatment with INORT on *Plumeria frangipani* cv Super round". Irrigation turn-over of 6 days. Legend: (CTRL) control; (MC): microorganisms; (AG): algae; (INORT): microorganisms+algae+inula

5. Conclusion

The trial showed that the use of a liquid biostimulant based on *Inula viscosa* was able to significantly improve the growth, vegetative and root biomass of *Plumeria frangipani*. The treatment also provides increased resistance to water stress and protection against plant attack by *Fusarium oxysporum*.

This highlights other interesting and innovative aspects of the use of this plant, which have already been highlighted in previous trials on various potted plant species. Given the importance of Iinula viscosa from a medicinal, pollination and biodiversity point of view, new agricultural experiments are very important as they could allow the development of new biofertiliser products that can be used in organic and sustainable farming systems.

Compliance with ethical standards

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Disclosure of conflict of interest

The author declares no conflict of interest.

Statement of ethical approval

The present research work does not contain any studies performed on animal/humans subjects.

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