World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(Research Article)

Plant extracts from mediterranean vegetation in the cultivation and defence of *Plectranthus coleidos* and *Plectranthus amboinicus*

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

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

World Journal of Advanced Research and Reviews, 2023, 18(02), 502–509

Publication history: Received on 02 April 2023; revised on 08 May 2023; accepted on 11 May 2023

Article DOI: https://doi.org/10.30574/wjarr.2023.18.2.0859

Abstract

Research objective: This research aims to evaluate the biostimulant potential of some Mediterranean vegetation extracts for the stimulation and defence of some *Plectranthus amboinicus* and *Plectranthus coleidos* plants. This is to increase knowledge on these plant extracts that could be used for the formulation of new plant biostimulants.

Materials and Methods: The experiments, which started in May 2022, were conducted in the greenhouses of CREA-OF in Pescia (Pt), Tuscany, Italy (43°54′N 10°41′E) on *Plectranthus amboinicus* and *Plectranthus coleidos*.

The experimental groups were: group control; group with oak macerated; group with myrtus macerated; group with wild fennel macerated; group with lentiscus macerated; group with inula macerated. On January 20, 2023, plant height, leaves number, vegetative weight, roots volume and length, the number of microorganisms in the substrate, plant dead number and pH were determined.

Results and Discussion: The experiment showed that the use of plant extracts as biostimulants can significantly improve vegetative and root growth and reduce plant mortality of *Plectranthus amboinicus* and *Plectranthus coleidos*. In general, a significant increase in plant height and number of leaves as well as vegetative and root biomass was observed in plants treated with all plant biostimulants, particularly with the treatments of macerated oak, macerated fennel and macerated inula. The treatments with macerated oak and macerated fennel were the best in all two Plectranthus species. Observations conducted on treatments with phytotherapeutic agents demonstrate their effectiveness as stimulants of the plants' self-defence mechanisms. In addition, some preparations appear to act as repellents and substances that make many pests lose their appetite or stimulus to feed. Their activity seems to be determined by the minerals and certain molecules present in the plants used to prepare the macerates. This is why some of these substances have been placed under observation by scientific research to verify their effects. However, the objective difficulty of obtaining reproducible results limits research and experimentation activities in this field and directs them towards the characterisation of the active ingredients that play a primary role in insecticide, fungicide, bactericide or herbicide action.

Conclusions: Herbal medicines are always used in aqueous form by performing the treatment with an atomiser or, more rarely, by adding the extract to irrigation water. The extract is obtained by steeping the herbs in water or by preparing an infusion or decoction. The test showed that the use of extracts obtained from plants by macerating Mediterranean shrub species can have biostimulant activity against aromatic succulents such as *Plectranthus amboinicus* and *Plectranthus coleidos*. Very interesting aspects especially with regard to the study of new formulations obtained from plants and applicable in organic farming.

* Corresponding author: Domenico Prisa

Keywords: Plectranthus; Microorganisms; Plant extracts; Biofertilizers; Rhizosphere

1. Introduction

In order to accommodate the predicted increase in world population from 7.7 billion to 9.6 billion by 2050, horticultural production must increase yield. Malnutrition affects nearly 1 billion people worldwide [1] and climate change threatens horticultural production stability [2]. Horticultural production is also affected by other biotic and abiotic stresses, such as drought, salinity, pests and diseases, and weed infestations. In addition, excessive reliance on chemical inputs to increase productivity may have adverse effects on human health and the environment, e.g. nitrification of waterways. Consumer preferences encourage the production of sustainable, healthy, and economical food, and increased costs are also a strong economic incentive to reduce inputs and simplify the husbandry of fruit crops [3]. In order to achieve sustainable horticultural production, alternative strategies need to be explored [4,5,6]. There are several strategies to achieve this goal, one of which is to use plant-derived biostimulants (PDBs) [7]. To increase production more sustainably, PDBs can help the agricultural sector address specific growth and reproduction issues. "Plant biostimulants" are defined by the European Biostimulant Industry Council as substances and/or microorganisms whose function, when applied to plants or the rhizosphere, is to stimulate natural processes to enhance/benefit nutrient uptake, nutrient efficiency, tolerance of abiotic stress, and crop quality." Various natural biostimulant compounds are classified according to their source and content (humic substances, seaweed extracts, amino acids containing products) [8], whereas products of plant origin contain free amino acids and polypeptides derived by extraction and/or enzymatic hydrolysis. Through improved plant physiological processes such as nutrient uptake, growth, and tolerance to abiotic stresses, PDBs can accelerate plant growth, protect plants from abiotic stresses, and/or improve nutrient use efficiency [9,10]. In high value production systems (e.g. greenhouse production, fruit, vegetable, and floriculture), PDBs are increasingly integrating to improve productivity and quality. As opposed to synthetic fertilizers derived from chemical industries, PDBs are naturally occurring products. Consequently, these products accumulate in the soil, contaminating water. In recent years, there has been an increased interest in using PDBs in horticulture to maintain yield, particularly in low input conditions in the horticultural sector [11], as they are natural products that are naturally found in all higher plants (although in varying concentrations and combinations). There have been several studies evaluating licorice root extract [12], legume-derived protein hydrolysate [13], lemon grass [14], garlic extract [15], and moringa leaf extract [16], among others.

1.1. The use of plants as biostimulants: some examples

Several examples illustrate the positive effects of PDBs on growth and yield, as well as physiological and biochemical characteristics of several horticultural crops. Studies have shown that licorice (*Glycyrrhiza glabra*) root extract improves: (a) growth and yield along with improved P content while mitigating the negative effects of salinity stress in beans [17], (b) nutritional and hormonal change in leaves of pears [18,19], (c) almond seedling vegetative growth [20,21], and (d) fennel growth, essential oil, and phytochemical content [22] when combined with fertilizant. Similar to carrot root extract, cowpea (*Vigna sinensis*) seedlings grown under salt stress showed increased growth, physiological processes, and phytochemical concentrations [23]. Using aloe leaf extracts, garlic leaf extracts, and lawsonia leaf extracts, as soil drench or foliar sprays, improved the growth and chemical concentration of dwarf umbrella tree (*Schefflera arboricola*) [24]. When green tea extract was applied at 150 ppm and garlic extract at 300 ppm, the effects of various leaf extracts (garlic and green tea, each at two different concentrations) on guar (*Cyamposis tetragonoloba*) were positive [25]. Seagrass aqueous extract was applied at 10-day intervals foliarly to salt-stressed tomatoes to mitigate the negative effects of salinity [26]. Several horticultural crops, including eggplants [27], snap beans [28], tomatoes [29] and olive trees [30], benefited from aqueous garlic extracts in terms of growth, physiological traits, and phytochemical content and composition. It has been shown that oak bark-derived biostimulants can improve grapevine quality [31] in terms of colour [32], polyphenol content [33], aroma [34] and overall quality [35]. Wine quality has also been reported to be improved by foliar application of vine shoot extracts and trimming waste from Airén vineshoots [36].

1.2. Research Objectives

This research aims to evaluate the biostimulant potential of some Mediterranean vegetation extracts for the stimulation and defence of some *Plectranthus amboinicus* and *Plectranthus coleidos* plants. This is to increase knowledge on these plant extracts that could be used for the formulation of new plant biostimulants.

2. Material and methods

The experiments, which started in May 2022, were conducted in the greenhouses of CREA-OF in Pescia (Pt), Tuscany, Italy (43°54′N 10°41′E) on *Plectranthus amboinicus* and *Plectranthus coleidos* (Figure 1A,1B). The plants were placed in ø 12 cm pots, 30 plants per thesis, divided into three replicas of 10 seeds each. All plants were fertilized with a controlled release fertilizer (1 kg m^{-3} Osmocote Pro®, 9-12 months with 190 g/kg N, 39 g/kg P, 83 g/kg K) mixed with the growing medium before sowing. The experimental groups were:

- Group control (CTR) (peat 80%+ pumice 20%), irrigated with water and substrate previously fertilized;
- Group with oak macerated (3 ml, twice a week) (OAK) (peat 80% + pumice 20%) and fertilised substrate;
- Group with myrtus macerated (3 ml, twice a week) (MYR) (peat 80% + pumice 20%) and fertilized substrate;
- Group with wild fennel macerated (3 ml, twice a week) (FEN) (peat 80% + pumice 20%) and fertilized substrate;
- Group with lentiscus macerated (3 ml, twice a week) (LEN) (peat 80% + pumice 20%) and fertilized substrate;
- Group with inula macerated (3 ml, twice a week) (INU) (peat 80% + pumice 20%) and fertilized substrate;

The plants were watered one time a day and grown for six months. Then, 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 leaching fraction. On January 20, 2023, plant height, leaves number, vegetative weight, roots volume and length, the number of microorganisms in the substrate, plant dead number and pH were determined.

2.1. Analysis methods

- pH: For pH measurement, 1 kg of the substrate was taken from each plant, and 50 g of the mixture was placed in a beaker containing 100 ml of distilled water. After 2 hours, the water was filtered and analyzed [13];
- Microbial count: directly determining total microbial count by microscopy cells contained in a known sample volume using counting chambers (Thoma chamber). The surface of the slide is etched with a grid of squares, with the area of each square known. Determination of viable microbial load after serial decimal dilutions, spatula seeding (1 ml) and plate counting after incubation [13];

2.2. Statistics

The experiment was carried out in a randomized complete block design. Collected data were analyzed 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 the LSD multiple-range tests ($P = 0.05$). Statistics and graphics were supported by the programs Costat (version 6.451) and Excel (Office 2010).

3. Results

The experiment showed that the use of plant extracts as biostimulants can significantly improve vegetative and root growth and reduce plant mortality of *Plectranthus amboinicus* and *Plectranthus coleidos*. In general, a significant increase in plant height and number of leaves as well as vegetative and root biomass was observed in plants treated with all plant biostimulants, particularly with the treatments of macerated oak, macerated fennel and macerated inula (Figure 1, 2 and 3) (Table 1-2). The treatments with macerated oak and macerated fennel were the best in all two *Plectranthus* species. There was also an increase in the number of leaves, 20.41 (OAK) and 15.22 (FEN), compared to the control with 8.11 in *Plectranthus amboinicus*, and 18.00 (OAK) and 15.20 (FEN) compared to the control with 7.60 in *Plectranthus coleidos*. The (OAK) and (FEN) treatments also significantly elongated the roots; OAK and FEN showed an average elongation of 1-1.5 cm more than the control in all two succulent species. There was also a significant reduction in the mortality of plants treated with the plant biofertilisers. Another effect was the increase in microbial biomass in the theses treated with plant extracts; the microorganisms probably utilise the metabolites contained in these plants as a nutrient substrate for their multiplication. Growing plants consequently benefit from an increase in microbial biomass in the rhizosphere, because plant-soil interactions are significantly increased, with a greater supply of nutrients. In general, there is no significant difference in the pH of the substrate, only the inula treatment in *Plectranthus coleidos* showed a significant lowering.

Table 1 Evaluation of the use of vegetables extract on the vegetative and root biomass of *Plectranthus amboinicus*

Groups	Plant height (cm)	Leaves number (n°)	Substrate total bacteria (Log CFU/g soil)	Vegetative weight (g)	Roots volume (cm³)	Roots length (cm)	Plants dead number(n°)	pH substrate
CTR	14.39 e	8.11 d	2.23 e	25.20 e	13.32 e	5.07 e	1.80 a	6.72 a
OAK	19.09 a	20.41 a	4.33 b	30.27 a	16.94 a	6.32 b	0.20 b	6.74 a
MYR	14.97 d	9.00 d	3.16 c	27.52 c	14.62 d	5.23de	0.20 b	6.82 a
FEN	18.64 b	15.22 b	4.65 a	28.29 b	15.46 c	6.93 a	0.00 b	6.74 a
LEN	14.63 de	12.63 c	2.92 d	26.66 d	14.70 d	5.39 d	0.00 b	6.68 a
INU	18.17 c	13.64 bc	3.29 c	26.96 cd	15.75 b	5.67 c	0.20 b	6.80 a
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).Legend: (CTR) control; (OAK) oak macerated;(MYR) myrtus macerated; (FEN) fennel macerated; (LEN) lentiscus macerated; (INU) inula macerated

Table 2 Evaluation of the use of vegetables extract on the vegetative and root biomass of *Plectranthus coleidos*

Groups	Plant height (cm)	Leaves number (n°)	Substrate total Bacteria (Log CFU/g soil)	Vegetative weight (g)	Roots volume (cm³)	Roots length (cm)	Plants dead number (n°)	pH substrate
CTR	15.55 e	7.60 e	2.17 c	22.36 d	11.81 d	4.40 d	1.41 a	6.82 a
OAK	19.65 a	18.00 a	4.27 a	25.71 a	14.63 a	6.47 a	0.20 b	6.66 ab
MYR	15.87 d	9.80 d	3.18 b	23.22 c	13.06 c	5.08 c	0.20 b	6.76 a
FEN	18.55 b	15.20 b	4.33 a	25.47 a	14.29 a	6.21 b	0.40 b	6.76 a
LEN	15.32 e	12.20 c	3.17 b	23.46 c	13.04 c	4.39 d	0.20 b	6.78 a
INU	16.90 c	13.80 b	3.17 b	23.97 b	13.52 b	5.15 c	0.20 b	6.48 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).Legend: (CTR) control; (OAK) oak macerated;(MYR) myrtus macerated; (FEN) fennel macerated; (LEN) lentiscus macerated; (INU) inula macerated

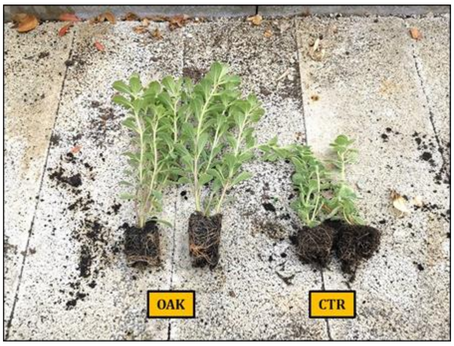

Figure 1 Comparison of macerated oak (OAK) and control (CTR) on the vegetative development of *Plectranthus amboinicus*

Figure 2 Comparison of macerated fennel (FEN) and control (CTR) on the vegetative development of *Plectranthus coleidos*

Figure 3 Comparison of macerated inula (INU) and control (CTR) on root development of *Plectranthus coleidos*

4. Discussion

In organic farming, protection from adversity is achieved by focusing mainly on prevention through agronomic and cultural practices whose aim is to create unfavourable conditions for pathogens and optimal conditions for plant growth. Favourable growing conditions are obtained by applying correct cultivation and agronomic practices: choice of hardy varieties, good organic matter content in the soil, good exposure, correct water availability, presence of mulch, appropriate crop rotation, presence of shrubs [37].

These favourable growing conditions allow plants to spontaneously activate their natural self-defence mechanisms and at the same time increase biodiversity through a richer presence of natural pathogen antogonists and a more diverse plant cover. Even under the best agronomic and environmental conditions, however, there is still the risk that adversity in general and pests in particular will cause serious damage to plants, so it is important to know how to recognise damage and the pests that cause it [38]. Herbal macerates, decoctions and infusions represent a system of plant stimulation and protection traditionally used in organic farming. They are mainly used to stimulate plants' natural defences and for their repellent and phagodeterrent effect [39]. The action performed by phytopreparations depends on the active ingredient content of the preparation and this in turn can change depending on many variables: the variety of plant used, the characteristics of the soil in which it grew, the climatic conditions in which it developed, the time at which it was harvested, the way in which the active ingredients were extracted (i.e. how the preparation took place), and the duration of the extraction [40]. Observations conducted on treatments with phytotherapeutic agents demonstrate their effectiveness as stimulants of the plants' self-defence mechanisms [16,17]. In addition, some preparations appear to act as repellents and substances that make many pests lose their appetite or stimulus to feed [21]. Their activity seems to be determined by the minerals and certain molecules present in the plants used to prepare the macerates. This is why some of these substances have been placed under observation by scientific research to verify their effects [24]. However, the objective difficulty of obtaining reproducible results limits research and experimentation activities in this field and directs them towards the characterisation of the active ingredients that play a primary role in insecticide, fungicide, bactericide or herbicide action. The reasons for this choice are also to be found in the commercial interest aroused by active molecules of natural origin. In fact, only from isolated molecules can one obtain registrable and marketable products [32]. Neem and pyrethrum are marketed as plant protection products and are of plant origin, but they no longer contain the phytocomplex, having been obtained by extraction and preparation methods that ensure a high concentration of the active ingredients, azadirachtin and natural pyrethrins respectively [26]. Phytopharmaceuticals of plant origin include mint oil, distributed as an anti-germinative for potatoes, clove oil, distributed as a post-harvest fungicide on apples and pears, and malaleuca oil, authorised only abroad as a fungicide against powdery mildew and botrytis. Studies have shown that the first two products are also effective in seed treatment. Trials have verified a decrease in the number of infected seeds, a reduction in the severity of symptoms manifested on plants and an improvement in seed emergence in the field [29]. Other trials have ascertained good results on post-harvest storage of Pomaceae and Drupaceae with treatments based on thyme essential oil and savory essential oil, but the development of commercial products is still at an early stage [19]. Instead, this trial showed that the use of macerates obtained from Mediterranean maquis plants can have a biostimulating and protective effect on aromatic succulent plants such as *Plectranthus amboinicus* and *Plectranthus coleidos*. In particular, extracts from oak and wild fennel, but also from viscous inula, myrtle and mastic have proven to be excellent plant growth promoters and deserve further investigation for their possible application in agriculture.

5. Conclusion

Herbal medicines are always used in aqueous form by performing the treatment with an atomiser or, more rarely, by adding the extract to irrigation water. The extract is obtained by steeping the herbs in water or by preparing an infusion or decoction. Steeping consists of leaving the herbs immersed in water at room temperature for a period ranging from a few hours to a few weeks. In this way, all the mineral salts and principles contained in the plant are extracted, including those that are thermolabile and very volatile. Due to their mineral salt content, macerates are similar to fertiliser products, so much so that in organic farming they are often used together with irrigation water to allow absorption by the roots. The test showed that the use of extracts obtained from plants by macerating Mediterranean shrub species can have biostimulant activity against aromatic succulents such as *Plectranthus amboinicus* and *Plectranthus coleidos*. Very interesting aspects especially with regard to the study of new formulations obtained from plants and applicable in organic farming.

Compliance with ethical standards

Acknowledgments

The research is part of the project **BIOMEDIMED**: Biostimulants obtained from plant extracts of the Mediterranean shrubland for the cultivation of aromatic and medicinal plants. We thank the company San Sebastiano al Vesuvio (Na) for funding the project.

Disclosure of conflict of interest

The author declares no conflict of interest.

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