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STUDIES ON FINDING RENEWABLE SOURCES OF PLANT BIOSTIMULATION – BIOACTIVE MACERATED EXTRACTS FROM WEEDS

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Abstract

In the scientific literature, there are several definitions of biostimulants. According to Chojnacka et al. 2014 which quotes on Vernieri et al. 2006 and Schmidt et al. 2008: biostimulants are environmental friendly, natural substances which are able to promote vegetative growth, mineral nutrient uptake, plant response to different pedoclimatic conditions and tolerance to abiotic stresses, biostimulants are organic materials that, when applied in small quantities, enhance plant growth and development such that the response cannot be attributed to application of traditional plant nutrients.
In this paper we present the results obtained from experiments that took place both in the laboratory, greenhouse and in the field. Through these experiments we wanted to test the capacity of bioactive macerated extracts from weeds, plants from the spontaneous flora of Romania, such as comfrey - *Symphytum officinale,* horsetail - *Equisetum arvense,* nettle – *Urtica dioica,* dandelion – *Taraxacum officinale , to stimulate the germination, growth and development of pepper seeds and tomato seedlings. We made the macerates from the plants from the spontaneous flora from our country according to the data found in the specialized literature with small differences and adaptations that we present to Materials and methods.*
The results obtained by us are encouraging because finding renewable sources of plant biostimulators is important in the context of modern agriculture, which seeks non-polluting solutions to replace polluting products - synthetic chemical fertilizers, pesticides, etc.

Key words*: bioactive macerated extracts,* comfrey - *Symphytum officinale,* dandelion – *Taraxacum officinale, germination,* horsetail - *Equisetum arvense,* nettle – *Urtica dioica, plant stimulation, renewable sources, weeds.*

INTRODUCTION

Anthropogenic climate change, namely climate alterations induced by human activities, is causing some issues to agricultural systems for their vulnerability to extreme events. Forecasts predict a global population increase in the near years that will exacerbate this situation, elevating the global demand for food. It will pose severe concerns in terms of natural resource usage and availability. Agriculture is one of the anthropogenic activities that will be more affected in the future. Climate extremes menace to affect the quantity and quality of crop production severely. Drought, water and soil salinity are considered among the most problematic factors that anthropogenic climate change will increase. This complex and worrying scenario requires the urgent implementation of sustainable measures which are capable of improving crop yield and quality, fostering the robustness and resilience of cropping systems (Del Buono, 2021).

Del Buono in his paper emphasizes the importance of finding quick solutions to counteract the negative anthropogenic effects on agriculture. Among the more current methodology, the use of natural plant biostimulants has been proposed to improve plant resistance to abiotic environmental stresses. The advantage of using these substances is due to their effectiveness in improving crop productivity and quality.

In the scientific literature, there are several definitions of biostimulants. According to Chojnacka et al. 2014 which quotes on Vernieri et al. 2006 and Schmidt et al. 2008 biostimulants are environmental friendly, natural substances which are able to promote vegetative growth, mineral nutrient uptake, plant response to different pedoclimatic conditions and tolerance to abiotic stresses, biostimulants are organic materials that, when applied in small quantities, enhance plant growth and development such that the response cannot be attributed to application of traditional plant nutrients.

Plant biostimulants attract interest in modern agriculture as a tool to enhance crop performance, resilience to environmental stress, and nutrient use efficiency. Plant biostimulants encompass diverse organic and inorganic substances (humic acids and protein hydrolysates) as well as prokaryotes (e.g., plant growth promoting bacteria) and eukaryotes such as mycorrhiza and macroalgae (seaweed). Global demographic pressure on agricultural production calls for novel and sustainable approaches toward satisfying the ever-growing demand for plant biomass destined for human food, animal feed, and energy production (Puglia et al., 2021). Conventional agricultural practice has relied overwhelmingly on non-renewable inputs of fertilizers and pesticides (Panfili et al., 2019). Although their introduction has allowed substantial progress for humankind, agro-chemicals also pose a serious, unresolved threat to human health and the environment. Moreover, supply and application of these inputs is becoming increasingly costly because of resource depletion and the increasing global demand for mineral fertilizers. Finally, the use of chemical inputs in agriculture is restricted by a tightening legal framework because of mounting public concern. Plant biostimulants can play a pivotal role in addressing sustainability challenges because they can reduce dependency on fertilizers, especially on off-farm chemical inputs. Moreover, plant biostimulants are also useful to improve yield and its stability under environmental stress (Chiaiese et al., 2018, De Pascale et al., 2018).

Preparation of plants, weeds in our case, for experimental purposes is an initial step and key in achieving quality research outcome. It involves extraction and determination of quality of bioactive constituents before proceeding with the intended biological testing. Although the extracts, bioactive fractions, or compounds obtained from plants are used for different purposes, the techniques involved in producing them are generally the same irrespective of the intended biological testing. The major stages included in acquiring quality bioactive molecule are the selection of an appropriate solvent, extraction methods, phytochemical screening procedures, fractionation methods, and identification techniques. The nitty-gritty of these methods and the exact road map followed solely depends on the research design. Solvents commonly used in extraction of plants are polar solvent (e.g., water, alcohols), intermediate polar (e.g., acetone, dichloromethane), and nonpolar (e.g., n-hexane, ether, chloroform). In general, extraction procedures include maceration, digestion, decoction, infusion, percolation, Soxhlet extraction, superficial extraction, ultrasound-assisted, and microwave-assisted extractions (Abubakar and Haque, 2020). Maceration is an extractive technique that is conducted at room temperature. It consists of immersing a plant in a liquid (water, oil, alcohol, etc.) inside an airtight container, for a variable time based on the plant material and liquid used. Before being processed, the plant must be properly washed and separated from foreign material such as topsoil, pebbles or rocks and materials non-suitable for extraction. The plant material can be used fresh or dry based on the desired product. In order to increase contact between the plant material being extracted and the liquid (solvent), the plant needs to be cut into small pieces. The pieces should not be too big, otherwise the solvent will not be able to penetrate the innermost cells. They also should not be reduced to powder; that would result in losing the volatile active ingredients (essential oils) contained inside the plant, and also losing the difficult separation by filtration of the plant material from the liquid used once maceration is completed. The solvent must be chosen based upon the chemical nature of the compounds contained within the plant. Solubility and the desired use of the extraction should be considered when choosing the solvent. That is, recognizing their solubility and the desired use of the extraction. Generally, alcohol is the most used substance because it is able to extract a greater part of the molecules (active ingredients) contained within the plant, including molecules which are hydrophilic, soluble in water, or lipophilic and therefore, soluble in oil or other organic solvents. One uses a vegetable oil when one wants to isolate only the lipophilic components (fats), while water is used to extract only hydrophilic ingredients. (https://albrigiinherba.com/contacts/extraction/maceration/)

MATERIALS AND METHODS

Making organic fertilizer based on macerated plants from spontaneous flora

In order to make ecological fertilizers based on macerates from plants from spontaneous flora, bioactive macerated extracts from weeds, we went through the following steps:

1. we chopped the plants (comfrey - *Symphytum officinale*, horsetail - *Equisetum arvense*, nettle – *Urtica dioica* and dandelion – *Taraxacum officinale* leaves) put them in a bucket, then added water and covered with a lid.
2. Mixing daily the maceration for aeration.
3. The mixture is left to soak for a few days to 1 week until the fermentation process ceases, a process that depends on the outside temperature.
4. After fermentation ceases, the plant material is allowed to decant and then it can be used as such or diluted for foliar applications to tomatoes, eggplants, peppers, etc., in the field, greenhouse, solarium or lab.

In vitro experiments

For in vitro experiment we used organic fertilizer based on bioactive macerated weed extracts in the Ecology and Microbiology laboratory of the F.I.F.I.M. within the U.S.A.M.V. Bucharest to demonstrate the power of stimulating the germination and development capacity of some capsicum seeds. The laboratory experiment was conducted in 2019 starting with March and was carried out as follows:

1. In two containers we put forest soil from Băneasa forest that contained microorganisms (bacteria, fungi, etc.) with a role in stimulating the growth and development of plants by producing enzymes, growth hormones and 100 capsicum seeds.
2. After sowing, the control was watered two or three times a week with sterile distilled water, and the sample was watered with the ecological fertilizer obtained by macerating the plants from the spontaneous flora, bioactive macerated extracts from weeds.

Determination of N, P, K concentrations of organic fertilizer obtained from plant macerates from spontaneous flora

Using the Hanna Instruments analysis kit, we determined the N, P, and K concentrations of the organic fertilizer obtained.

In vivo experiments

The in vivo experiments took place in a vegetable garden in Tamasi village, Corbeanca commune, Ilfov county. The aim of the in vivo experiments was to achieve an organically grown tomato crop and to highlight the ability of plant bioactive macerated extracts from weeds, herbal macerates from spontaneous flora, to feed plants properly and to fights phytopathogenic fungi of the species Phytophthora infestans that cause manna to plants in tomatoes, a disease that is frequently recorded in solariums, greenhouses and in the field and that causes significant damage to crops, the affected plants must be removed and destroyed.

The tomato variety chosen for this experiment was the Cristal F1 hybrid, which is an early tomato variety, with undetermined growth, intended for cultivation in greenhouses and solariums, but which we cultivated in open space in the garden, not in protected space, to test its ability to adapt to outside conditions. For planting we used F1 Crystal seedlings, which were planted in the garden between April 23 and April 25, 2020.

RESULTS AND DISCUSSIONS

Making organic fertilizer based on macerated plants from spontaneous flora

Figure 1. Mixing chopped plants with water

Figure 2. Daily mixing for aeration of the macerate

Figure 3. The accumulation of foam on the surface of the container marks the maximum of the maceration fermentation process

Figure 4. Cessation of the fermentation process

Figure 5. Decantation of plant material

Figure 6. Preservation of organic fertilizer based on bioactive macerated weed extracts in recyclable containers

In vitro experiments

Figure 7. Germination of Control pepper seeds

Figure 8. Germination of pepper seeds Sample

Figure 9. Control capsicum seeds in forest soil containing microorganisms with a role in stimulating plant growth and development

Figure 10. Sample of capsicum seeds treated with organic fertilizer based on macerates from plants from spontaneous flora and forest soil containing microorganisms with a role in stimulating plant growth and development

Figure 11. Control (left) and Sample (right)

Determination of N, P, K concentrations of organic fertilizer obtained from plant macerates from spontaneous flora

Using the Hanna Instruments analysis kit, we determined the N, P, and K concentrations of the organic fertilizer obtained. The images below show the results of the laboratory tests using the Hanna Instruments colorimetric analysis kit for the qualitative determination of N (nitrogen), P (phosphorus), K (potassium or potassium).

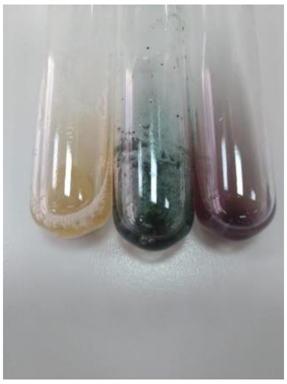

Figure 12. Determination of N, P, K from right to left

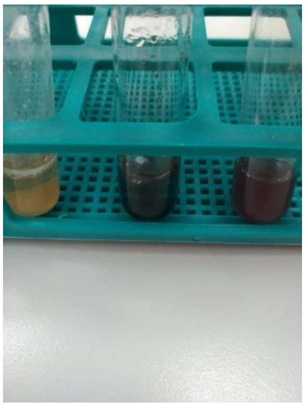

Figure 13. The stand with the test tubes for determining N, P, K from right to left

The results of the analyzes N, P, K show us that the ecological fertilizer based on plant macerates from the spontaneous flora obtained is rich in these macroelements essential for the healthy and harmonious growth and development of plants.

By testing the germination capacity of capsicum seeds and the growth and development of seedlings we demonstrated the effectiveness of organic fertilizer, observing from the photos of the experiments the big differences between Control containing only forest soil with beneficial microorganisms in the category PGPM (Plant Growth Promoting Microorganisms) and Sample containing in addition to the same type of soil and organic fertilizer based on plant macerates from spontaneous flora.

In vivo experiments

Carrying out cultural treatments - application of vegetable fertilizer and phytosanitary treatments

We used the fertilizer thus obtained in the garden for foliar spraying on tomato plants of the variety Cristal F1 diluted 1: 2, 1 part fertilizer and 2 parts water and undiluted to water the soil from each nest at the base of the package of tomato plants.

We performed a leaf spray and one at the base of the plants 5 days after the time of planting the seedlings, then when the flowers began to appear, then when the plants began to bear fruit and a last treatment when the fruits began to ripen. So in total we performed four spray treatments.

For the protection of plants against phytopathogenic fungi of the species Phytophthora infestans that cause late blight of tomatoe, a particularly dangerous disease with very great economic damage, we used Bordeaux juice (bouille-Bordelaise), which is a highly effective cupric fungicide containing 20% copper metallic and 80% neutralized copper sulfate. Being perfectly neutralized, micronized, it has a long effectiveness and is one of the phytosanitary products accepted in organic farming.

We applied phytosanitary treatments as preventive foliar to tomato plants 10 days after planting the seedlings, then immediately after the first signs of attack appeared on the leaves of some tomato plants. We prepared a Bordeaux juice solution using 75 g of blue powder mixture in 10 liters of water. With the solution thus obtained we sprayed all the plants with the help of vermorel.

Next we will present the experimental field with Cristal F1 tomatoes grown in the garden in an uncovered space in an ecological system.

Figure 14. Cristal F1 tomato plants grown organically (the first 4 floors with the fruits formed on each plant can be observed)

Figure 15. Detail tomato bunches on the “first floor”

Figure 16. The first signs of attack with Phytophthora infestans (top right - leaf affected)

Figure 17. Application of phytosanitary treatment with Bordeaux mixture at the first signs of attack with late blight of tomato

Figure 18. Growing and ripening tomatoes from the first floor of the Cristal F1 variety grown organically

Figure 19. The harvest was very rich due to the treatment with bioactive macerated extracts from weeds

CONCLUSIONS

Our experimental studies testing the action of bioactive macerated extracts from several weeds, under open-field, greenhouse and lab conditions, have demonstrated that they stimulate germination, seedling growth, shoot, and root biomass in crops such as tomato and pepper.

In the coming years, research efforts should focus on: elucidating the bioactive macerated extracts from weeds × plant species × environment interaction, in order to select optimal combinations; optimizing application parameters (timing, mode, rate of application, and plant developmental stage); quantitative and qualitative characterization of microbial communities as modulated by bioactive macerated extracts from weeds utilized as plant biostimulants; determining the persistence of effects subsequent to bioactive macerated extracts from weeds foliar application; the impact of climatic (radiation and relative humidity) and plant morphological factors (cuticle thickness and leaf permeability) on the effectiveness of bioactive macerated extracts from weeds.

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