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Chabatitic Zeolites With Earthworm Humus Added To The Growing Media To Improve Germination and Growth of Horticultural Plants

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Abstract- In order to improve the quality and protection of horticultural crops, the capacity of chabazitic zeolites with earthworm humus at different percentages added to the growing medium was evaluated. The five experimental groups in cultivation were: i) group without chabazite and earthworm humus; ii) group with (chabazite/earthworm humus 70-30) 10%; iii) group with (chabazite/earthworm humus 70-30) 20%; iv) group with (chabazite/earthworm humus 50-50) 10%; v) group with (chabazite/earthworm humus 50-50) 20%. All the plants of *Lactuca sativa*, *Brassica juncea red*, *Chycorium intybus*, *Eruca vesicaria*, *Brassica rapa japonica* have shown a significant improvement in the vegetative and radical growth in the thesis treated with chabazitic zeolites added with earthworm humus. In particular the theses with (chabazite/earthworm humus 70-30) 20% and (chabazite/earthworm humus 50-50) 20% were the most performing and showed a significant improvement of all the agronomic parameters analysed. The study in question is therefore of particular interest, especially when the horticultural species in question are grown in greenhouses where biotic and abiotic stresses are very important and finding sustainable methods to reduce their effects is essential.

***Keywords*:** Horticulture; Sustainable applications; Potted plants; Rhizosphere, Microorganisms

I. INTRODUCTION

Natural Zeolites are a mineral family composed by 54 different species chemically defined as "hydrated allumino-silicates of alkaline and alkaline earth elements" and structurally belonging to the tectosilicates. Due to their crystal chemistry, zeolites show physical-chemical peculiarities such as high and selective cation exchange capacity (CEC), reversible dehydration, selective molecular absorption, and catalytic behaviour. Therefore, rocks containing more than 50% of zeolites (zeolitites) are widely and profitably utilized in the purification of municipal, zootechnical and industrial wastewaters, as additive in animal nutrition, agriculture and floriculture [1].

Because of both the presence of the zeolites and texture of the rocks, zeolitites exhibit high (130-200 meq/100g) and selective (mainly for NH_4 and K^+) cation exchange capacity, reversible dehydration, permeability, and high water retention, which are all useful in agricultural, horticultural and floricultural applications. Commercial use of natural zeolites is still in its infancy, but more than 300,000 tons of zeolite-rich tuff is extracted annually in the United States, Japan, Bulgaria, Hungary, Italy, Yugoslavia, Korea, Mexico, Germany, and the Soviet Union [2]. The pronounced selectivity of zeolite for large cations, such as ammonium and potassium, has also been exploited in the preparation of chemical fertilizers that improve the capacity of retention of nutrients in soils promoting a slower release of these elements for absorption by plants, in rice fields, where losses by runoff of 50% of nitrogen supplied are frequent [3].

Earthworm humus is a fine-sized, peat-like soil improver material, microbiologically stable and active, with a low C/N ratio, high porosity and high water retention capacity, containing many nutrients for the plants, which forms are easily available for their nutrition. This material results from the interactions between earthworms (Lumbricidae) and microorganisms which occur during the degradation of the organic substance and is composed mainly of faecal residues (casts) of earthworms which build the starting organic matrix for their needs metabolics. It is a compound very rich in organic substance characterized by high rates of this has a very positive effect on the availability of nutrients for the plants, particularly with regard to ammonia and nitrate forms [4].

Earthworm humus is an accelerated process of bio-oxidation and stabilization of organic matter, promoted by the interaction between earthworms and microorganisms. It is an eco-sustainable process that does not require high technological levels for its process and can also be used for the treatment of organic residues from separate collection. It is a complex process both at a biological and ecological level because, although earthworms are the primary factor of the process (from which it takes its name), the presence of complex interactions between organic matter, microorganisms, earthworms and other soil organisms (meso and macrofauna) are the basis of the process of fragmentation, biooxidation and stabilization of organic matter [5,6].

II. RELATED WORK

The main objective of potted plants is the use of substrates and biostimulant that can reduce the use of peat and increase plant quality As the price of these materials has been rising in recent years as a result of rising energy costs that are reflected in the entire process of production, preparation and transport to farmers. The alternative materials used often create problems for plants related to rooting or water and salt stress. Zeolites commonly utilized in agriculture for the cultivation of horticultural and ornamental crops [7,8] and for the reduction of NH^{4+} content in the liquid manure in the pig farms [9], could resolve in part this problem. These minerals, added to peat or to other organic compost at 20% content, are practical to use, easy to mix to the soil or to other substrates, also for soilless cultivation. The active nutrients and water content result always available to plant and the adsorbed fertilizing elements are safe from the risk of run-off due to rain or irrigation [10].

Earthworm humus has a great potential especially used for the formulation of potted substrates [11]. Researchers evaluated the effect of different sources of N (manure, ammonium nitrate and earthworm humus) and obtained higher yields on *Raphanus sativus* L. and *Capsicum annum* L. with earthworm [12]. Earthworms influence the structure of the compost by forming macropores, which allow oxygen to enter, and alsoincrease the stability of the humus and itsability to retain water [13]. On the occasion of the passage through the worms, not only organic matter but also the mineral components that serve as food are subject to the following to digestive enzymes and a grinding process [14]. A improved grass grow tharound worm waste indicates increased availability of plant nutrients, with perhaps nitrogen being the mineral that is subject to the greatest influence [15]. With in its digestive tract, soil material under goes transformations, with decomposition of organic matter and availability of nutrients for plants.

Earthworm humus significantly stimulates the growth of a number of plant species including several horticultural [16] crops such as peppers [17], garlic [18], strawberries [19], sweet corn [20] and beans [21]. In addition, there were positive results on some aromatic and medicinal plants [22], cereals such as sorghum and rice [23], tree crops such as banana and papaya [24], and on ornamental plants such as geranium [25], marigold [26] and poissettia [27]. Positive effects have also been found on forest species such as *Acacia* sp., *Eucalyptus* sp. and *Pinus* sp. [28].
In order to improve the quality and protection of horticultural crops, the capacity of chabazitic zeolites with earthworm humus at different percentages added to the growing medium was evaluated.

III. METHODOLOGY

The experiments, started in December 2019, were conducted in the greenhouses of CREA-OF in Pescia (Pt), Tuscany, Italy (43°54′N 10°41′E) on *lactuca sativa, brassica juncea red (Mizuna), chicorium intybus, eruca vesicaria, brassica rapa japonica*. The plants were placed in ø 12 cm pots; 30 plants per thesis, divided into 3 replicas of 10 plants each. All plants were fertilized with a controlled release fertilizer (3 kg m-3 Osmocote Pro®, 6 months with 190 g/kg N, 39 g/kg P, 83 g/kg K) mixed with the growing medium before transplanting. The five experimental groups in cultivation were:

- Group without chabazite and earthworm humus (CTRL) (peat 70% + pumice 30%), irrigated with water and substrate previously fertilized;
- Group with chabazitic zeolites added with earthworm humus 10% (CHAMUS10) (peat 60%+ (chabazite/earthworm humus 70-30) 10% + pumice 30%), irrigated with water and substrate previously fertilized;
- Group with chabazitic zeolites added with earthworm humus 20% (CHAMUS20) (peat 60%+ (chabazite/earthworm humus 70-30) 20% + pumice 30%), irrigated with water and substrate previously fertilized;
- Group with chabazitic zeolites added with earthworm humus 10% (CHAHUM10) (peat 60%+ (chabazite/earthworm humus 50-50) 10% + pumice 30%), irrigated with water and substrate previously fertilized;
- Group with chabazitic zeolites added with earthworm humus 20% (CHAHUM20) (peat 60%+ (chabazite/earthworm humus 50-50) 20% + pumice 30%), irrigated with water and substrate previously fertilized;

All chabazite products were supplied by Balco Greenline of Sassuolo (MO). The chabazitic- zeolites had the following characteristics: 1) qualitative-quantitative mineralogical analysis (% by weight with standard deviations in brackets) carried out by X-ray powder diffractogram according to the RIETVELDRIR methodology [29]: chabazite 66.2 (1.0); phillipsite 2.4 (0.5); mica 5.6 (0.6); K-feldspar 10.3 (0.8); pyroxen 2.2 (0.5); volcanic glass 13.3 (1.5); 2) Total zeolithic content (%): 68.6 (1.3), of which 66.2 due to chabazite and 2.4 from phillipsite. Cation exchange capacity (in meq/g with standard deviation in brackets) determined using the methodology described in Gualtieri et al. (1999) [30]: 2.15 (0.15) of which 1.42 due to Ca, 0.04 to Mg, 0.05 to Na and 0.64 to K. The chabazite with added microorganisms had a microbial count of 4.2 x 106 ufc/g of zeolites. The pH of the zeolite was 6.9. For the experimentation of plant growth was used ø 0-3 mm.

For the product based on earthworm humus, ONUS CLT® by Centro Lombricoltura Toscano of Parco S. Rossore (PI) was used. Onus® is a product obtained from the digestion of earthworms (*Eisenia fetida*, *Eisenia andrei*) from bovine manure, horse manure and sheep manure. Percentage values on dry matter: total nitrogen 1,7; organic nitrogen 1,6; organic carbon 23,7; C/N 13,9; organic matter 47,4; extractable organic matter 58,7; humified organic matter 81,6; pH 7,6.

The plants were watered 2 times a week and grown for 4 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 March 11, 2020, percentage of seed emergence, total leaf area per plant (mm2), primary root lenght (mm), number of leaves per plant, number of lateral roots, aerial part and root system (mg), were recorded.

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 the plants of *lactuca sativa*, *brassica juncea red*, *chycorium intybus*, *eruca vesicaria*, *brassica rapa japonica* have shown a significant improvement in the vegetative and radical development in the thesis treated with chabazitic zeolites with earthworm humus. The experimentation also showed that the theses (CHAMUS20) and (CHAHUM20) are those where there is a greater increase in agronomic parameters.

In particular in *lactuca sativa* (Table 1) the percentage of seed emergence (E%) was 63.00 in (CHAHUM20) and 62.60 in (CHAMUS20) compared to 47.00 in the untreated control. The thesis (CHAMUS20) with 365.78 mm^2 was the best in total leaf area per plant. The thesis (CHAHUM20) was the best with regard to primary root lenght, number of leaves per plant and number of lateral roots compared to all other treated and control theses. The thesis (CHAMUS20) and (CHAHUM20) were the best in terms of weight of aerial parts and radical system.

In *brassica juncea red* (Table 2) there was a significant improvement in the seed emergence rate in the thesis (CHAMUS20, CHAHUM20 and CHAMUS10). The thesis (CHAMUS20) and (CHAHUM20) were the best in total leaf area per plant, number of leaves per plant, weight of radical system. While the thesis (CHAHUM20) was the best for primary root lenght and (CHAMUS20) for aerial part weight. All the theses were significantly better than the control per number of lateral roots.

In *chycorium intybus* (Table 3) the thesis (CHAMUS20) had been the preferred one with regard to the percentage of seed emergence and the weight of the aerial part. The thesis (CHAMUS20) and (CHAMUM20) were the best for primary root lenght, number of leaves per plant and number of lateral roots. The thesis (CHAMUS20) was instead distinguished for having increased total leaf area per plant.

In eruca vesicaria (Table 4) the thesis (CHAHUM20) was the preferred one for seed emergence percentage, number of lateral roots, aerial parts and weight of the root system. The thesis (CHAHUM20) and (CHAMUS20) were the best for total leaf area per plant, primary root length, number of leaves per plant.

In *brassica rapa japonica* (Table 5) the thesis (CHAHUM20) was the better for percentage of seed emergence, primary root lenght, number of leaves per plant, radical system weight. The thesis (CHAHUM20) and (CHAMUS20) were the best for total leaf area per plant and number of lateral roots. The thesis (CHAMUS20) was the best for aerial part weight.

Experiments have demonstrated the ability of chabazitic zeolite added to earthworm humus, when placed in the growing medium, to significantly increase the quality of horticultural plants such as *lactuca sativa, brassica juncea red, chycorium intybus, eruca vesicaria* and *brassica rapa japonica* in terms of seed germination rate and increase in vegetative and root biomass. This improvement may have been influenced both by the ability of zeolites to increase the availability of water and fertilizers when the plants need them, and by the nutritional and biostimulating capacity of earthworm humus, which is also influenced by the rich presence of microbial flora that can improve the structure of the substrate, facilitate the absorption of nutrients, reduce the presence of biotic and abiotic stress. [31,32].

Zeolites in fact have a high cation exchange capacity, an interesting feature is the possibility that the internal cavities of their structure can accommodate calcium, magnesium or sodium ions, and that these ions can be easily replaced by ammonium and potassium ions for which zeolites have a high affinity. They are considered to be real molecular sieves and thanks to these particular properties are used in various sectors such as water purification, petrochemical industry, animal breeding and as biostimulants in horticulture [33,34].

Zeolites, in fact, once introduced in growing substrates or in open field, can increase the quality of plants, retaining water and fertilizers and making them available when needed.
The use of earthworm humus can stimulate flowering, increase plant and root biomass and plant production and growth. However, there are also studies that report that the use of earthworm humus can decrease plant growth or even cause plant death. The variability of the effects of earthworm humus may depend on the culture system in which it is incorporated, which is highly dependent on the basic organic matrix, the earthworm species used, the production processes and age. It provides a reservoir of macro and micronutrients for plants [35].

In this experiment the theses with (chabazite/earthworm humus 70-30) 20% and (chabazite/earthworm humus 50-50) 20% were the most performing and showed a significant improvement of all the agronomic parameters analysed.

Table 1 - evaluation of chabazitic zeolites with earthworm humus on agronomic characters on plants of *lactuca sativa*

Lactuca sativa	E%	TLAP (mm2)	PRL (mm)	NLP (n°)	NLR (n°)	AP (mg)	RS (mg)
CTRL	47,00 c	224,90 d	88,06 e	7,60 e	5,80 b	821,44 d	41,58 b
CHAMUS10	56,40 b	308,28 c	96,20 d	12,40 d	6,60 b	871,38 c	44,28 ab
CHAMUS20	62,60 a	365,78 a	108,04 c	17,40 b	7,00 b	903,64 a	49,06 a
CHAHUM10	57,80 b	292,02 c	116,02 b	15,00 c	5,80 b	893,16 b	44,50 ab
CHAHUM20	63,00 a	347,38 b	133,98 a	20,20 a	8,40 a	905,06 a	44,68 a
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: E% = percentage of seed emergence; TLAP = total leaf area per plant (mm2); PRL = primary root length (mm); NLP = number of leaves per plant; NLR = number of lateral roots; AP = Aerial parts; RS = Radical system.
Treatments: CTRL=control; CHAMUS10=(chabazite/earthworm humus 70-30) 10%; CHAMUS20=(chabazite/earthworm humus 70-30) 20%; CHAHUM10=(chabazite/earthworm humus 50-50) 10%; CHAHUM20= (chabazite/earthworm humus 50-50) 20%.

Table 2 - evaluation of chabazitic zeolites with earthworm humus on agronomic characters on plants of *brassica juncea*

Brassica juncea	E%	TLAP (mm2)	PRL (mm)	NLP (n°)	NLR (n°)	AP (mg)	RS (mg)
CTRL	43,20 d	84,16 c	44,80 d	4,80 c	3,80 c	89.08 d	24,38 c
CHAMUS10	54,80 b	94,48 b	54,74 c	6,60 b	6,00 ab	95,14 c	35,06 b
CHAMUS20	59,20 a	98,82 a	61,28 b	7,80 a	6,40 ab	103,64 a	40,12 a
CHAHUM10	52,00 c	93,08 b	54,94 c	6,60 b	5,60 b	94,02 c	35,12 b
CHAHUM20	57,20 ab	98,34 a	65,02 a	8,40 a	6,80 a	99,66 b	40,58 a
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: E% = percentage of seed emergence; TLAP = total leaf area per plant (mm2); PRL = primary root length (mm); NLP = number of leaves per plant; NLR = number of lateral roots; AP = Aerial parts; RS = Radical system.
Treatments: CTRL=control; CHAMUS10=(chabazite/earthworm humus 70-30) 10%; CHAMUS20=(chabazite/earthworm humus 70-30) 20%; CHAHUM10=(chabazite/earthworm humus 50-50) 10%; CHAHUM20= (chabazite/earthworm humus 50-50) 20%.

Table 3 - evaluation of chabazitic zeolites with earthworm humus on agronomic characters on plants of *chycorium intybus*

Chycorium intybus	E%	TLAP (mm^2)	PRL (mm)	NLP (n°)	NLR (n°)	AP (mg)	RS (mg)
CTRL	50,40 d	186,52 e	76,60 d	15,00 c	4,00 c	718,24 e	32,10 c
CHAMUS10	57,60 b	196,58 c	83,84 b	20,60 b	5,40 b	751,78 c	37,22 b
CHAMUS20	58,80 b	211,48 a	88,64 a	23,20 a	6,20 a	766,22 b	42,28 a
CHAHUM10	54,00 c	193,52 d	79,06 c	19,80 b	4,80 b	738,86 d	37,48 b
CHAHUM20	66,80 a	203,92 b	88,88 a	25,00 a	6,60 a	781,78 a	43,98 a
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: E% = percentage of seed emergence; TLAP = total leaf area per plant (mm2); PRL = primary root length (mm); NLP = number of leaves per plant; NLR = number of lateral roots; AP = Aerial parts; RS = Radical system.
Treatments: CTRL=control; CHAMUS10=(chabazite/earthworm humus 70-30) 10%; CHAMUS20=(chabazite/earthworm humus 70-30) 20%; CHAHUM10=(chabazite/earthworm humus 50-50) 10%; CHAHUM20= (chabazite/earthworm humus 50-50) 20%.

Table 4 - evaluation of chabazitic zeolites with earthworm humus on agronomic characters on plants of *eruca vesicaria*

Eruca vesicaria	E%	TLAP (mm^2)	PRL (mm)	NLP (n°)	NLR (n°)	AP (mg)	RS (mg)
CTRL	46,20 d	75,22 d	44,42 d	4,40 c	1,80 d	73,82 d	32,18 d
CHAMUS10	54,00 c	83,82 c	51,38 b	5,40 b	3,60 c	76,28 c	36,90 c
CHAMUS20	56,80 b	94,34 a	54,70 a	6,40 a	4,60 b	82,48 b	43,14 b
CHAHUM10	45,00 d	87,26 b	48,88 c	5,60 b	4,20 bc	83,08 b	35,78 c
CHAHUM20	59,00 a	95,40 a	53,64 a	6,80 a	5,60 a	86,36 a	45,18 a
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: E% = percentage of seed emergence; TLAP = total leaf area per plant (mm2); PRL = primary root length (mm); NLP = number of leaves per plant; NLR = number of lateral roots; AP = Aerial parts; RS = Radical system.
Treatments: CTRL=control; CHAMUS10=(chabazite/earthworm humus 70-30) 10%; CHAMUS20=(chabazite/earthworm humus 70-30) 20%; CHAHUM10=(chabazite/earthworm humus 50-50) 10%; CHAHUM20= (chabazite/earthworm humus 50-50) 20%.

Table 5 - evaluation of chabazitic zeolites with earthworm humus on agronomic characters on plants of *brassica rapa japonica*

Brassica rapa j.	E%	TLAP (mm^2)	PRL (mm)	NLP (n°)	NLR (n°)	AP (mg)	RS (mg)
CTRL	45,20 d	86,58 c	46,20 d	5,00 d	2,20 c	89,64 d	33,88 e
CHAMUS10	53,40 c	92,40 b	53,08 c	6,20 c	3,20 b	91,68 c	39,04 c
CHAMUS20	59,40 b	97,54 a	58,66 b	7,20 b	4,60 a	96,20 a	44,68 b

CHAHUM10	53,80 c	88,86 c	53,14 c	6,60 bc	3,40 b	88,72 d	36,26 d
CHAHUM20	62,60 a	96,58 a	61,22 a	8,20 a	4,40 a	94,78 b	47,74 a
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: E% = percentage of seed emergence; TLAP = total leaf area per plant (mm2); PRL = primary root length (mm); NLP = number of leaves per plant; NLR = number of lateral roots; AP = Aerial parts; RS = Radical system.
Treatments: CTRL=control; CHAMUS10=(chabazite/earthworm humus 70-30) 10%; CHAMUS20=(chabazite/earthworm humus 70-30) 20%; CHAHUM10=(chabazite/earthworm humus 50-50) 10%; CHAHUM20= (chabazite/earthworm humus 50-50) 20%.

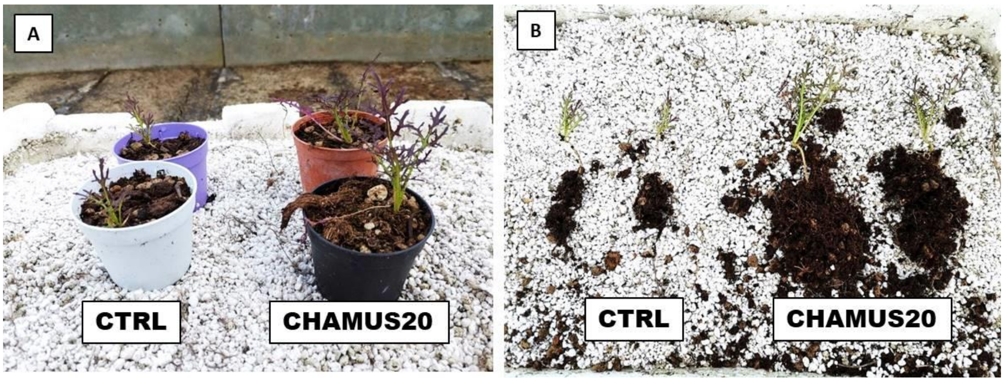

Figure 1 - Effect of chabazitic zeolites with earthworm humus on vegetative and roots biomass of *brassica juncea* red. Legend: (CTRL): control; (CHAMUS20): (chabazite/earthworm humus 70-30) 20%

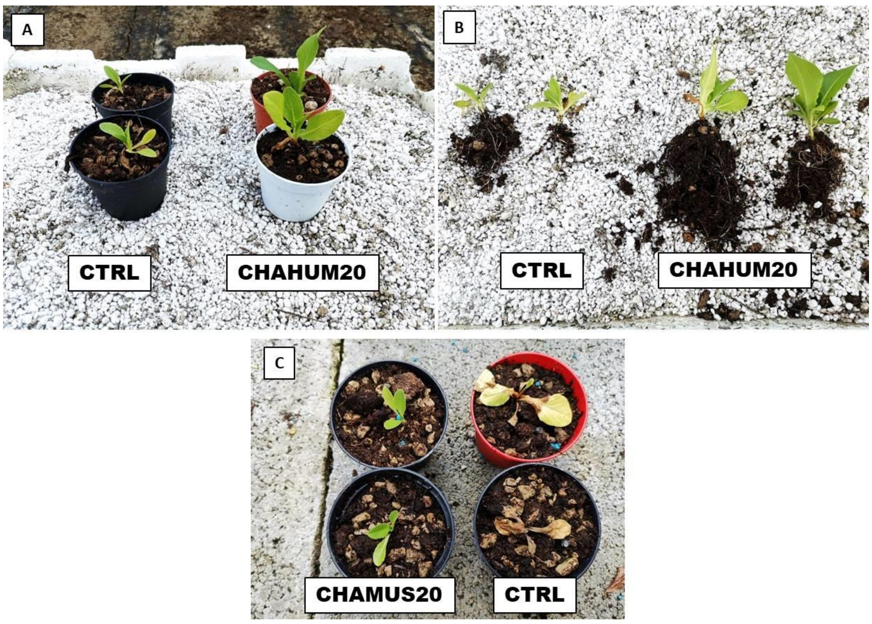

Figure 2 – Effect of chabazitic zeolites with earthworm humus on vegetative and roots biomass of *lactuca sativa*. Legend: (CTRL): control; (CHAHUM20): (chabazite/earthworm humus 50-50) 20%; (CHAMUS20): *(chabazite/earthworm humus 70-30) 20%*

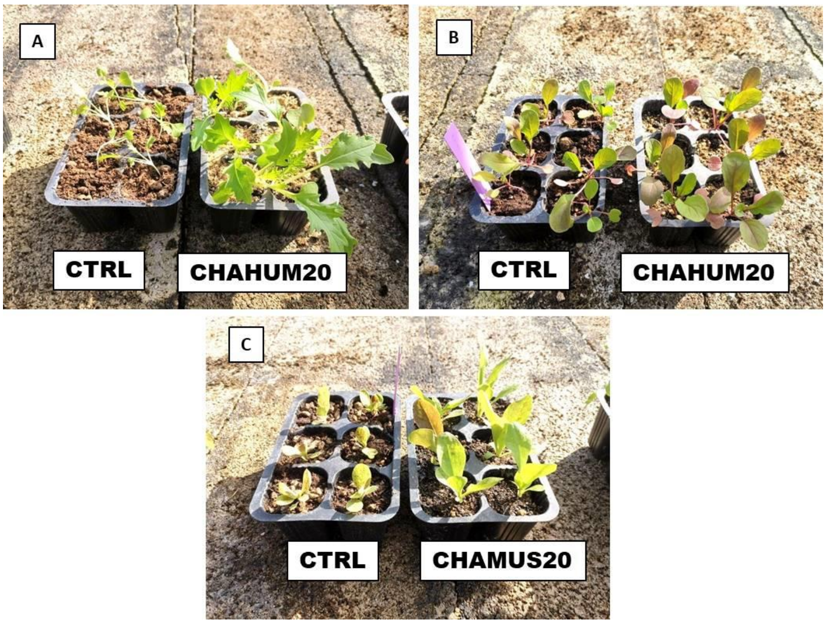

Figure 3 – Effect of chabazitic zeolites with earthworm humus on vegetative biomass of *brassica rapa japonica* (A), *eruca vesicaria* (B) and *chycorium intybus* (C). Legend: (CTRL): control; (CHAHUM20): (chabazite/earthworm humus 50-50) 20%; (CHAMUS20): (chabazite/earthworm humus 70-30) 20%

V. CONCLUSION AND FUTURE SCOPE

The test showed that the use of chabazitic zeolites with earthworm humus significantly increases the germination and agronomic quality of *lactuca sativa*, *brassica juncea red* (Mizuna), *chicorium intybus*, *eruca vesicaria*, *brassica rapa japonica*. In particular the theses with (chabazite/earthworm humus 70-30) 20% and (chabazite/earthworm humus 50-50) 20% were the best in improving the vegetative and root development of plants. These mixtures can therefore be easily added to the growing medium and improve it nutritionally, structurally and biologically.

The study in question is therefore of particular interest, especially when the horticultural species in question are grown in greenhouses where biotic and abiotic stresses are very important and finding sustainable methods to reduce their effects is essential.

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