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Chabazitic zeolite in the cultivation and spray protection of *Vitis vinifera*

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Abstract

Research objective: This article aims to highlight how chabazite zeolite can lead to improved growth and protection of vine plants, in particular the benefits it can bring to crops in terms of plant development, reduced incidence of fungal diseases and pesticide reduction.

Materials and Methods: The experiments, which began in January 2023, were conducted in the CREA-OF greenhouses in Pescia (Pt), Tuscany, Italy (43°54′N 10°41′E) on *Vitis vinifera* cv 'Moscato giallo' and 'Passerina' plants. The plants were placed in pots with a diameter of 16, 50 plants per 3 replications, for a total of 150 seedlings per experimental thesis. The first trial on *Vitis vinifera* involved the following theses (irrigated and fertilized): i) peat 70% + pumice 30%; ii) peat 70% + pumice 20% + zeolite chabazite 10%; iii) peat 70% + pumice 10% + chabazite zeolite 20%. The second trial included spray treatments with micronized zeolite on the leaves to assess the control of diseases such as *Botrytis cinerea*, *Oidium tuckeri* and downy mildew. The trial included the following theses (irrigated and fertilized):

- Control with treatment with water sprayed on the leaves every 10 days;
- Control with copper-based treatment 1 kg/hl (copper oxychloride + lime) + folpet + sulphur 200g/hl, every 10 days;
- Treated with chabazite zeolite 1.5 kg/hl + 250 g/hl (copper oxychloride) + sulphur, every 10 days.

On 17 July 2023, plant height, vegetative weight, root volume and length, leaf area and the number of microorganisms in the substrate were determined. The number of plants affected by *Botrytis cinerea*, *Oidium tuckeri* and downy mildew was also assessed.

Results and Discussion: The experiment showed that the use of zeolite has chabazite added to the cultivation substrate at the rate of 10-20% can effectively improve the vegetative and root growth of *Vitis vinifera*. Furthermore, when micronized chabazite zeolite is sprayed on the leaves, it can better contain diseases such as *Botrytis cinerea*, *Oidium tuckeri* and Downy mildew, compared to the use of copper and sulphur alone. According to the researchers, they have extensively researched chemicals that have excellent antifungal activity against a wide variety of fruit and vegetable diseases but can also be safely applied to crops. This has led them to find out that copper-containing zeolites have excellent fungicidal activity while exhibiting no problematic phytotoxicity. Plant pathogenic fungi are controlled by using crystalline zeolite (in particular chabazite), an agricultural and horticultural fungicide.

Conclusions: In recent years, rapid population growth and urbanization have limited the agricultural area's ability to produce more crops. To perform more efficient agricultural activities, higher nutrient application rates are needed and more efficient water irrigation systems must be used. The problem, however, is that a substantial amount of nutrients might be washed out, polluting water resources and reducing product yields if high rates of fertilizers are applied to

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soils. For this reason, a cost-effective, pervasive, and green solution is essentially required to increase crop yields. Zeolites have been extensively investigated to enhance agricultural productivity.

Keywords: Alternative substrates; Zeolites; Plant growth; Vitis; Rhizosphere

1. Introduction

The *Vitaceae* genus has been present in Europe since the latest Eocene (around 40 million years ago), while *Vitis vinifera*, at least judging by the fossil finds so far, is dated to the Pleistocene, around one million years ago [1]. However, according to various authors, the presence of vines can be traced back to even earlier eras, from 65 to 100 million years ago. The first systematic classification of *Vitis vinifera* of the Linnean type dates back to 1774, by the German botanist Johann Friedrich Gmelin, and is practically the same as today. The vine belongs to the order *Rhamnales*, family *Vitaceae* or *Ampelidaceae*, to which the two subfamilies *Lecoldeae* and *Ampelideae* belong [2]. The genera *Ampelopsis*, *Cissus*, *Parthenocissus*, *Ampelocissus* and *Vitis* belong to the subfamily *Ampelideae*. *Vitis vinifera sylvestris*, from which today's cultivated *Vitis vinifera* sativa is derived, is frequently found in forests and can be found, like other vines, on rocks and in various situations in its natural state, for example in the mountains up to 1,500 metres above sea level [3]. The vines cultivated today originate from the east, but they did not come to the west passively, i.e. they were not transported on purpose, but were subjected to various types of mutation and natural cross-breeding over the centuries [4]. Furthermore, it has recently been shown by Mario Sanges and collaborators that, for example, certain grape varieties were present spontaneously on the island of Sardinia and were not brought there either by the Phoenicians or by other peoples. To the genus *Vitis* belong the subgenera *Muscadinia* and *Euvitis* [5]. The *Muscadinia* subgenus is only used for experimentation, as it is resistant to cryptogamic diseases, phylloxera and nematodes; it is therefore hybridised with *Vitis vinifera* with the aim of obtaining rootstocks resistant to the aforementioned pathogens. *Vitis rotundifolia*, characterised by large berries, is indigenous to the United States and Mexico where it is also cultivated. The subgenus *Euvitis*, which has 19 double chromosomes, has pyriform grape seeds [6].

1.1. The annual biological cycle of the vine

The annual cycle of the vine is expressed through two phenological phases: the vegetative cycle and the reproductive cycle [7]. The vegetative cycle comprises the growth period, the processing period and the winter rest period. The vegetative cycle consists of vegetative growth and begins with weeping, which consists of the outflow of colourless, transparent sap from pruned branches, in varying quantities, from a few decilitres to a few litres for the most vigorous plants [8]. Planting lasts 15 to 20 days, takes place between the end of March and the beginning of April and indicates the vegetative recovery of the vine after the winter rest [9]. Weeping is a consequence of the rapid increase in the activity of the root system and the consequent considerable increase in the absorption of water and mineral salts. It must be borne in mind that the root system continues its activity even during the winter period and reaches its maximum activity in spring, during weeping and sprouting, and then in autumn [10,11]. The high concentration of mineral salts in the roots and then in the stem and branches causes a strong increase in osmotic pressure, which is the cause of the release of sap, i.e. weeping. To prevent weeping, pruning is done early so that the wound from the pruning cut has time to heal [12]. The first process that clearly demonstrates the vegetative recovery of the plant is the budding, which consists of the swelling of the bud, the opening of the bracts and then the opening of the buds, i.e. the emergence of the shoot. With the opening of the buds the weeping phenomenon ceases. The treatment of the vineyard with hydrogen cyannamide, in particular, favours sprouting and a general anticipation of the various phenological phases [13,14]. Once sprouting is complete, growth begins, which proceeds rather slowly, following the increase in atmospheric temperature, soil humidity and hormonal action, reaching maximum development in conjunction with flowering; it then slows down with the summer drought and ends towards the end of July when the sprout has reached its maximum and final length. Instead, the enlargement of the sprout is the work of the cambium, as occurs in all plant species [15]. The shoots that develop the most are the vertical ones that suffer more from apical dominance than the curved ones, the vine being an acrotonous plant. Various factors intervene in the regulation of shoot growth, above all the hormones produced by the vine itself exert a clear influence [16].

1.2. Zeolite and zeolitite in agriculture

During the 1909 Nobel Prize winning season, the German chemist Fritz Haber converted nitrogen gas from the atmosphere, which is abundant but non-reactive, into reactive nitrogen that can be oxidized or volatilized. In the decades that followed, many industries began to process tons of industrial ammonia into synthetic fertilizers because of Haber's idea [17]. A German chemist, Carl Bosch, developed a method to exploit Haber's idea industrially. The invention of Haber-Bosch is regarded as one of the most important discoveries in the field of population development [18]. The Green Revolution was defined as a process that influenced agricultural practice between 1940 and 1970, and which, as a result of the use of genetically engineered plant varieties, fertilizers, pesticides, water, as well as new technological and mechanical means, boosted agricultural production throughout the world [19,20]. With synthetic fertilizers, farmers have been able to cultivate poor soils or continuously exploit the same soil with the same crop to increase production. As a result of the ability to produce crop after crop without allowing nutrients to regenerate naturally in the soil, population growth has been favoured, with the world's population growing from 1.6 to 6 billion by the end of the 21st century [21]. Farming practices currently in use, which don't naturally regenerate nutrients, have a high cost: fertilizers, potassium, nitrogen, and phosphorus are artificially added to the soil for the purpose of obtaining increasingly good harvests, but they inevitably lead to environmental problems [22]. There are serious environmental consequences to the widespread use of agropharmaceuticals (particularly highly soluble copper salts) as foliar treatment against phytophagous and micropathogenic insects that severely damage crops [23]. As a result of their leaching by rainwater and irrigation water, underlying soils become polluted, resulting in loss of fertility. It has been described in several papers in the literature [24,25]. However, very few scientific papers describe the use of zeolites in agriculture and how farmers can benefit from their use [26]. This article aims to highlight how chabazite zeolitite can lead to improved growth and protection of vine plants (**Figure 1**), in particular the benefits it can bring to crops in terms of plant growth, reduced incidence of fungal diseases and pesticide reduction.

Figure 1 Details of the substrates and plants used in the experiment at CREA-OF

2. Material and methods

The experiments, which began in January 2023, were conducted in the CREA-OF greenhouses in Pescia (Pt), Tuscany, Italy (43°54′N 10°41′E) on *Vitis vinifera* cv 'Moscato giallo' and 'Passerina' plants.

The plants were placed in pots with a diameter of 16, 50 plants per 3 replications, for a total of 150 seedlings per experimental thesis.

All plants were fertilised with a controlled-release fertiliser (2 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 transplanting. The trial was divided into two parallel trials with different substrates. The first trial on *Vitis vinifera* involved the following theses (irrigated and fertilised)

- Peat 70% + pumice 30% (CTRL)
- Peat 70% + pumice 20% + zeolite chabazite 10% (ZEO10)
- Peat 70% + pumice 10% + chabazite zeolite 20% (ZEO20)

The second trial on *Vitis vinifera* included spray treatments with micronized zeolite on the leaves to assess the control of diseases such as *Botrytis cinerea*, *Oidium tuckeri* and downy mildew. The trial included the following theses (irrigated and fertilised)

- Control with treatment with water sprayed on the leaves (CTRL) every 10 days;
- Control with copper-based treatment 1 kg/hl (copper oxychloride + lime) + Folpet + sulphur 200g/hl (CTRL2), every 10 days
- Treated with chabazite zeolite 1.5 kg/hl + 250 g/hl (copper oxychloride) + sulphur (ZEOCHAB), every 10 days.

The plants were watered once a day and grew for seven months. The plants were drip-irrigated. Irrigation was activated by a timer whose programme was adjusted weekly according to the weather conditions and the leaching fraction.

On 17 July 2023, plant height, vegetative weight, root volume and length, leaf area and the number of microorganisms in the substrate were determined. The number of plants affected by *Botrytis cinerea*, *Oidium tuckeri* and downy mildew was also assessed.

2.1. Methods of analysis

Microbial count: direct determination of the total microbial count by microscopy of the cells contained in a known volume of sample 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.

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 zeolite has chabazite added to the cultivation substrate at the rate of 10-20% can effectively improve the vegetative and root growth of *Vitis vinifera* (Table 1). Furthermore, when micronized chabazite zeolite is sprayed on the leaves, it can better contain diseases such as *Botrytis cinerea*, *Oidium tuckeri* and Downy mildew, compared to the use of copper and sulphur alone (Table 2).

Generally speaking, in the first experiment with zeolite added to the growing medium on *Vitis vinifera*, a significant increase in plant height was observed with 20% zeolite (ZEO20), followed by theses with 10% zeolite and the untreated control (CTRL) (Figure 2).

All the theses were significantly better than the control (CTRL) with peat and pumice. The same trend was observed with regard to vegetative growth, root growth (Figure 3), leaf area and root length. An increase in microbial biomass was also observed in the substrates of the theses with zeolite compared to the control.

In the second trial concerning the use of micronized zeolite spray for plant protection the application of chabazite zeolite in combination with copper and sulphur (ZEOCHAB) resulted in a significant improvement in plant protection against *Botrytis cinerea*, *Oidium tuckeri* (Figure 4) and Downy mildew compared to the use of copper and sulphur alone (CTRL2). The trend was the same for all disease types.

Table 1 Evaluation of the use of different substrates on *Vitis vinifera*

Groups	Plant height (cm)	Substrate total bacteria (Log CFU/g soil)	Vegetative weight (g)	Roots volume (cm^3)	Roots length (cm)	Leaf area (mm^2)
CTRL	22.94 c	1.64 c	56.87 c	36.41 c	9.30 c	300.53 c
ZEO10	26.06 b	3.89 b	60.38 b	39.34 b	11.36 b	321.84 b
ZEO20	29.27 a	4.63 a	63.83 a	42.92 a	13.63 a	330.22 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).Legend: (CTRL) peat 70% + pumice 30%; (ZEO10) peat 70% + pumice 20% + zeolite chabazite 10%; (ZEO20) peat 70% + pumice 10% + chabazite zeolite 20%

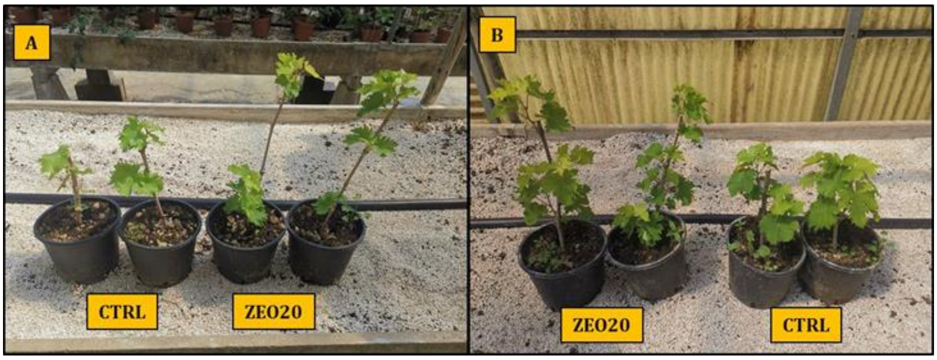

Figure 2 Comparison of the thesis with peat 70% + pumice 30% (CTRL) and peat 70% + pumice 10% + zeolite chabazite 20% (ZEO 20) on the vegetative growth of the varieties 'Passerina' (A) and 'Moscato giallo' (B)

Figure 3 Comparison of the thesis with peat 70% + pumice 30% (CTRL) and peat 70% + pumice 10% + zeolite chabazite 20% (ZEO 20) on root growth of the 'Passerina' variety

Table 2 Evaluation (number of plants with disease symptoms) of the use of different spray treatments with micronized chabazite zeolite on the control of certain fungal diseases

Groups	***Botrytis cinerea* (n°)**	***Oidium tuckeri* (n°)**	**Downy mildew (n°)**
CTRL	5.21 a	13.24 a	15.11 a
CTRL2	3.62 a	6.41 b	7.83 b
ZEOCHAB	1.88 b	3.44 c	5.81 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).Legend: (CTRL) treatment with water sprayed on the leaves; (CTRL2) control with copper-based treatment 1 kg/hl (copper oxychloride + lime) + Folpet + sulphur 200g/hl; (ZEOCHAB) chabazite zeolite 1.5 kg/hl + 250 g/hl (copper oxychloride) + sulphur

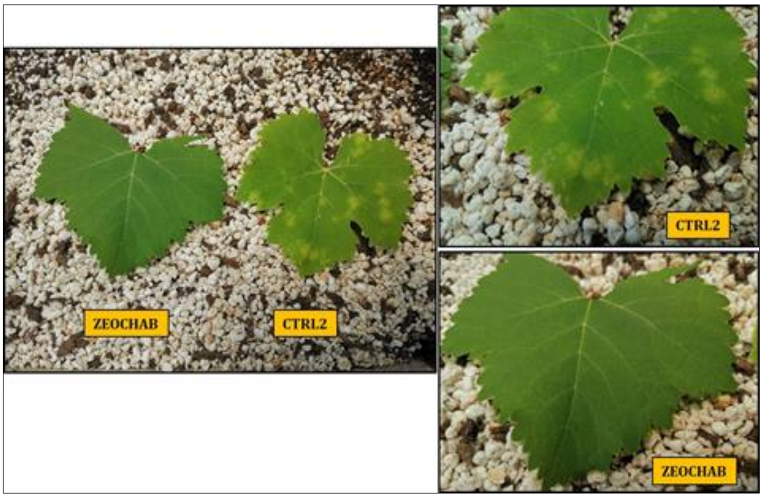

Figure 4 Comparison of the theses with copper treatment 1 kg/hl (copper oxychloride + lime) + Folpet + sulphur 200g/hl (CTRL2) and zeolite chabazite 1.5 kg/hl + 250 g/hl (copper oxychloride) + sulphur (ZEOCHAB) in protection against *Oidium tuckeri*

4. Discussion

In general, zeolites are natural minerals made up of more than 50 different minerals with various physical and chemical properties [27]. These minerals are mined or synthesized throughout the world. In essence, zeolites are hydrated aluminosilicates made from sodium, potassium, calcium, and magnesium cations [28]. Typically, zeolites are found in unmetamorphosed sedimentary rocks, and they are especially common in volcanic rocks covered in white material. Synthetic zeolite is manufactured or synthesized by breaking and/or making chemical bonds through one or more chemical reactions [29,30]. The compounds are described as hydrated aluminum-silate complexes of alkali and alkaline earth cations with a three-dimensional structure framework of SiO_4 and AlO_4 tetrahedra [31]. They combine rarity, beauty, complexity, and unique crystal habits. Environmental, agricultural, and health applications depend on the unique physical and chemical properties of zeolite [32]. There are many environmental applications for zeolite, including water and wastewater treatment, pollution control, ammonium ion removal, and air and gas purification. Agricultural applications include fungicides and herbicides, aquaculture, soil conditioning, feed supplements, and slow-release insecticide carriers [33-35]. As a result of their limited effectiveness against a narrow range of diseases and phytotoxicity to crops, many fungicides are not satisfactory [36]. Consequently, it has been desired to develop high-performance chemicals that have little phytotoxicity and are effective at low doses [37,38]. According to the researchers, they have extensively researched chemicals that have excellent antifungal activity against a wide variety of fruit and vegetable diseases but can also be safely applied to crops [39-41]. This has led them to find out that copper-containing zeolites have excellent fungicidal activity while exhibiting no problematic phytotoxicity [42,43]. Plant pathogenic fungi are controlled by using crystalline zeolite (chabazite, clinoptilolite, phillipsite and faujasite), an agricultural and horticultural fungicide [44,45]. Kaoline clay, attapulgite clay, bentonite, terra alba, pyrophillite, talc, diatomaceous earth, calcite, corn stalk powder, walnut shell powder, urea, ammonium sulfate, synthetic hydrated silicon dioxide, calcium lignosulfonate, sodium laury sulfate, polyoxyethylene sorbitan monooleate and water are examples of inert carriers. Agricultural and horticultural fungicides can be obtained from copper ion exchange of aluminum silicate [46]. In reality, the copper-containing aluminosilicate does not have a strong fungicidal activity per copper content and it cannot compensate for the defect of the conventional inorganic copper-containing chemicals. In order to be highly effective at a low dosage rate of copper, a chemical with a high fungicidal activity per copper content has been developed. Copper content in crystalline zeolite results in extremely high activity. In most cases, the present fungicide may be applied by any method or combination of methods used by those skilled in the art, such as foliage application, soil treatment, seed disinfection, etc [47]. Agricultural and horticultural fungicides use it as a active ingredient, and the dosage rate varies depending on the crop to be protected, disease to be controlled, disease outbreak intensity, preparation methods, application time, weather conditions, and copper content. As a result of zeolites' unique characteristics, their application can enhance water use efficiency (WUE) by increasing soil water content. Infiltration and hydraulic conductivity are two of the most important soil physical properties improved by zeolites [48]. According to extensive research, soil amendment using zeolitic materials improves water holding and prevents it from deep percolating, which reduces water use in agricultural applications. Many studies have investigated their impact on soil hydraulic properties [49]. Zeolite can improve the WHC of light-textured soils. Natural zeolites show a cation exchange capacity (CEC) of between 100 and 200 cmol (+) kg^{-1}. Zeolite is a silicate mineral whose characteristics differ from other silicate minerals such that it has spacious pores and channels within its structure. Natural zeolites are loaded with cations such as sodium (Na^+), potassium (K^+), and calcium ($Ca2^+$) [50]. These compounds possess a number of significant and well-known properties. The first one is a high CEC, which is much greater than that of soils, the second one is a large amount of free water within their structural channels, and the third one is a high surface area for adsorption [51]. This experiment carried out on *Vitis vinifera* shows both an improvement in plant growth in the theses treated with zeolite added to the substrate due to increased water and nutrient uptake and microbiological interactions in the plant, and a significant improvement in protection from phytopathologies when micronized zeolite is sprayed on the leaves. Besides having a repellent effect against insects, zeolite has a disruptive and dehydrating effect. The product causes problems with the respiratory system, burns the exoskeleton, problems in flight and reflects light. In addition, chabazite zeolite, having a rough structure, causes adherence problems on the leaf.

5. Conclusion

In recent years, rapid population growth and urbanization have limited the agricultural area's ability to produce more crops. To perform more efficient agricultural activities, higher nutrient application rates are needed and more efficient water irrigation systems must be used. The problem, however, is that a substantial amount of nutrients might be washed out, polluting water resources and reducing product yields if high rates of fertilizers are applied to soils. For this reason, a cost-effective, pervasive, and green solution is essentially required to increase crop yields. Zeolites have been extensively investigated to enhance agricultural productivity. Physical behaviors of soils can be affected by zeolites. The most notable impacts are that they reduce soil bulk density and increase soil porosity. These effects, in combination with their high internal pore volume, can help improve water retention. Furthermore, the open network of the zeolites structure can lead to the formation of new routes for water movement, which can consequently improve infiltration rate and saturated hydraulic conductivity. In agricultural applications, natural zeolites can be exploited for their outstanding chemical and physical properties, such as their high capacity for cation exchange and affinity for K^+ and NH^{4+}. A thorough evaluation of the risk of leaching toxic surfactants loosely attached to the zeolite surface should be conducted before any commercial applications can be made. Natural, synthetic, and modified zeolites can be used to prepare nitrogen, potassium, and sulfur slow-release fertilizers based on their ion exchange properties. A combination of zeolite ion exchange and mineral dissolution (similar to phosphate rock) can also be used to control and slowly release phosphorous. The impacts of zeolite application on soil physical and chemical properties are generally determined by a variety of experimental conditions, including zeolite type and application rate, method of application, soil texture, structure, zeolite particle size, and salinity of the water. A zeolite amendment may have a greater impact on coarse-textured soils than on fine-textured soils.

Compliance with ethical standards

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