# Vegetable culture vs. climate change Innovative solutions Part 1. Research on the chemical analysis of Buzau white onion bulbs cultivated using diatomite and *Trichoderma*

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#### ABSTRACT – REZUMAT

## Vegetable culture vs. climate change Innovative solutions Part 1. Research on the chemical analysis of Buzau white onion bulbs cultivated using diatomite and *Trichoderma*

In the context of intense and forced industrialization, agricultural overexploitation and pesticide pollution, attempts are being made to find organic alternatives for fertilizing and herbicide crops and products with an insecticidal effect that does not give bio-resistance over time. Given the desire of people to consume organic products, there is an increasing need to expand ecologically grown areas in order to make them available to consumers. For this purpose, it was decided to carry out research on the chemical analysis of Buzau white onion bulbs, treated with diatomite and Trichoderma, correlating them with the impact on the plants under study. Among the experimental variants, the best polyphenol content was found in variant V2 treated with 52.5 g of diatomite/repetition, compared to the untreated control. The experiments took place within S.C.D.L. Buzau, in the pedoclimatic conditions of the area, the results being available for informing growers and consumers. The paper is part of a complex research project in which these are solutions to reduce the negative climate impact in agriculture, especially in vegetable crops. Conventional and unconventional, but as much as possible sustainable solutions, including textile structures, organic diatomite, eco-friendly equipment generates an innovative instrument vs. climate change effects in vegetable crops. Part 2 of this research presents the textile solution in the context of the approached topic.

Keywords: diatomite, onion bulbs, Trichoderma spp., chemical analysis, textile nets for vegetable protection

#### Culturi legumicole vs. schimbări climatice Soluții inovative Partea 1. Cercetări privind analiza chimică a bulbilor de ceapă albă de Buzău cultivată utilizând diatomită și *Trichoderma*

În contextul industrializării intense și forțate, a supraexploatării agricole și a poluării cu pesticide, se încearcă găsirea unor alternative organice pentru fertilizarea și erbicidarea culturilor și a produselor cu efect insecticid care să nu genereze bio-rezistență în timp. Având în vedere dorința oamenilor de a consuma produse ecologice, este din ce în ce o mai mare nevoia de a extinde suprafețele cultivate ecologic pentru a le pune la dispoziția consumatorilor. În acest scop, s-a decis efectuarea de cercetări privind analiza chimică a bulbilor de ceapă albă de Buzău, tratați cu diatomită și Trichoderma, corelându-i cu impactul asupra plantelor studiate. Dintre variantele experimentale, cel mai bun conținut în polifenoli a fost găsit în varianta V2 tratată cu 52,5 g de diatomită/repetiție, comparativ cu martorul netratat. Experimentele au avut loc în cadrul S.C.D.L. Buzău, în condițiile pedoclimatice din zonă, rezultatele fiind disponibile pentru informarea cultivatorilor și a consumatorilor. Lucrarea face parte dintr-un proiect complex de cercetare în care acestea sunt soluții pentru diminuarea impactului climatic negativ în agricultură, în special în culturile de legume. Soluțiile convenționale și neconvenționale, pe cât posibil soluții durabile, incluzând structuri textile, diatomită organică, echipamente ecologice, generează un instrument inovator față de efectele schimbărilor climatice în culturile de legume. Partea a 2-a acestei cercetări prezintă soluția textilă în contextul subiectului abordat.

Cuvinte-cheie: diatomită, bulbi de ceapă, Trichoderma spp., analize chimice, plase textile pentru protejarea legumelor

# INTRODUCTION

In the current context of the cultivated agricultural surfaces expanding in an ecological system, organic strategies for crops fertilization/herbicides alternatives and pathogens plant management by biological means [1], represents the most important direction as a friendly alternative environment strategy for sustainable agriculture. This global direction is extremely important because about one-third of produced

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crops are destroyed by pests and diseases [2]. In an attempt to reduce these inconveniences and to increase onion production, researchers and agricultural producers are in a permanent correlation research-development for suitable and effective as possible pest control management strategies. Most often synthetic fungicides are used to control fungal infections in onion crops. Because the onion bulb is affected by fungal pathogens in the soil, chemical reducing agents are used, such as Granosan, which is capable to decline fungal disease by 77% and leads to increased onion productivity with 106%, or carbendazim and antracol, which are very effective in controlling basal rot of onion [1].

Although the development of chemical pesticides is advanced, it was demonstrated a significant negative impact on the environment and population health [2, 3]. New strategies of non-pathogenic microorganism had been developed, that acts as a bio-control agent e.g., Trichoderma spp. [2]. This microorganism is safe for humans, highly effective, with multiple benefits against plant pathogenic fungi [1, 3] and also it is used to increase abiotic and biotic factors plant resistance [4]. This Trichoderma biological alternative to classic synthetic pesticides includes a wide range of species [3] that can be easily isolated, being found inaccessible sources, such as soil microflora of various ecosystems, agricultural fields, forests, and wetland areas in all climatic zones [4]. Horticultural researches demonstrated that Trichoderma species is a good plant growth promoter applied in many crops, especially in onion [5, 6], garlic (Allium sativum) [7], rice [8], potatoes and tomatoes cultures [9], beans, onions or peppers [4], providing major benefits in farming systems, such as the mitigation of biotic and abiotic stresses [4, 10].

Allium cepa (Alliaceae) represents a global economically important horticulture crop, due to its protective food and special nutritive value [11]. However, the onion white root and bulb are very vulnerable to pathogens factors, like fungal diseases, caused by the sclerotium-forming fungus Sclerotium cepivorum [7], or Fusarium, Pythium, Rhizoctonia, Sclerotinia, Botrvtis and Verticillium phatogens [7] and also to foliar factors [1], that conduced inevitably to Alliaceae yield and quality reduction. This is the situation of many countries that are fighting this big problem, such as Brazil, where the onion production is not sufficient to fulfil internal demand, due to low productivity [7], or Nepal where the rise cultures are seriously affected by the climate and soil quality. In this context, Trichoderma inoculation as a seedling treatment can help to enhance rice production and productivity [10]. In India, where Alternaria porri (Ellis) Neerg. is one of the destructive onion diseases, more than 70% yield losses were reported. In this case, the inhibitory effect of Trichoderma species viz., T. harzianum, T. pseudokoningii and T. virens on mycelial growth and spore germination of A. porri were used [11]. In vitro studies were reported an efficient Trichoderma control by reducing the incidence of disease to 20-53%, using dry leaf biomass of

Withania somnifera in combination with Trichoderma harzianum by controlling onion basal rot disease [1]. Furthermore, the same microorganism could be a new biocontrol agent in the strategy concepts against some pathogen factors like Fusarium oxysporum and F. solani that damping-off of onion seeds, improving significantly the germination phenomena, by coating the seeds and spraying the seedbeds with a Burkina Faso native Trichoderma suspension [6]. On the other hand, studies on pathogens in rice crops treated with friendly herbicide showed that Trichoderma applied treatment increased the yields by 24%, compared with organic culture, and with 52% compared with non-organic one. The beneficial effect of Trichoderma treatment was different in intensity, depending on the variety, respectively 26-41% higher than classified crops [10]. This aspect is found to be very important from an economic point of view and especially for intelligent ecological management that will lead exclusively to significantly increased production yields. A friendly alternative for harmful chemical variants [12] in biological control of commercial crops is diatomite or diatomaceous earth. Reported data has shown an effective role as a fertilizing and insecticide agent. Diatomiceous earth and its compounds are considered conventional natural agents by desiccation comportment against insect pests [13].

This is a non-toxic material for crops and human health, with great environmental compatibility [12, 13]. Its effectiveness as a bio-agent is given by the nature of the chemical composition and by the source, respectively a silicon dioxide fossilized skeletons (up to 90%), alumina and ferric oxide [12]. In the recent years this inert natural insecticide powder [12, 13, 14] and its fungistatic effects [12] had spread to many agricultural crops affected by extern factors, such as Spodoptera exigua [13], Rhyzopertha dominica, Sitophilus oryzae (L.) (Coleoptera Curculionidae) [15], Sitophilus granaries [14], nymphs of Blattella germanica [16], or Trialeurodes vaporariorum [17]. Diatomaceous earth as bioinput, fertilization agent was tested on important commercial crops like corn (Zea mays), bean (Phaseolus vulgaris L.), carrot (Daucus carota L.) and yellow potato (Solanum phureja) [17]. Studies have shown that the benefits of the crop are due to the combination of versatile diatomite with the presence of silica in the soil. So, the diatomite might be a very good bio-fertilizer depending on soil quality, respectively by water regimes and nutrient availability [18]. Until 2016, a few data regarding the availability of diatomite as fertilizing or insecticide agent in the horticultural areas of Romania are available. In this context, Thanassiou et al., in 2016, [15] performed a comparative study between Romanian and Greece diatomaceous earth, against S. oryzae, R. dominica, T. castaneum and O. surinamensis, observing the significantly better effects of Romanian diatomaceous earth (Patarlagele, Urloaia and Adamclisi sources).

In vitro efficacy was tested against PyriSec as a standard product. The test was conducted under a controlled climate and under natural feed (wheat grains) Sitophilus granarius L. (Coleoptera: Curculionidae) treatment, and the population's ability to reproduce was observed. Thus, after 60 days, the diatomaceous earth (Patarlagele source, Romania) has lockdown the insects' reproduction capacity with 100% efficiency, shortly after the administration. On the other hand, Urloaia and Adamclisi samples (Greece sources) had a block-level under 80% [14]. Therefore, the intensity effect of diatomaceous earth on agricultural crops pests is closely related to its behaviour and source. Bacterial diseases and Fusarium proliferatum represent a global problem that affects especially Allium genus (garlic, and more often onion crops). Onion bulb internal decomposition during maturation and curing, prior to harvest stages was observed. To reduce these inconveniences, diatomaceous earth-chlorine was used as a local onion culture treatment against pathogenic microorganisms [19]. The purpose of our research was to study the bio-fertilizer and herbicidal diatomite and Trichoderma treatments effect on white onion crops by the field cultivation technique.

The aim of the study consists in correlating the experimental data productivity and results/observations generated by the physic-chemical analyses of white onion products, with the impact and influence of the organic treatment applied, in relation to the pedoclimatic conditions of the Buzau area (Romania).

## EXPERIMENTAL

## Materials

In this study, there were used the following:

- white onion seedling (CSD biological category);
- covered solarium, S.C.D.L. Buzau type;
- granulated NPK-S 20/20/0-13 chemical fertilizer complex (Agropolic Chim., Bulgaria);
- diatomite suspension (CMC/diatomite powder);
- Vermorel pump type;
- Fusilade forte, Syngenta.

For all analysis performed, high purity reagents were used, and for the determination and studding of the parameters presented above, basic equipment was used, respectively a spectrophotometer, an analytical balance and a Memert oven.

## Methods

The white onion culture was performed in the experimental field of S.C.D.L. Buzau. In 2019 this central location was the main Menuet bean forerunner culture area for diatomaceous earth crops experimental surface.

The culture was established on  $1600 \text{ m}^2$ , for these research experiments being allocated 10 furrows of 30 m length, modelled at 1.40 m and with a canopy opening of 94 cm. From this surface, on a length of 2.5 m on the ends and one furrow on the side, there were eliminations, in order to avoid the edge effect.

The white onion seedling was executed in a covered solarium.

When it had 55 days from emergence:

- the planting was done on furrows, 4 rows per furrow, grouped in pairs, with between 50–60 plants/ each row of repetition;
- the planting scheme was 10 + 15 + 54 + 15 + 10 cm/furrow, which means about 220 plants/repetition  $-7 \text{ m}^2$  and about 880 plants/variant  $-28 \text{ m}^2$ .

The planting step was done at 10 cm between plants/row, with twice per week drip irrigation, in the vegetation period. A rehearsal had a length of 5m and a width of 1.4 m, meaning 7 m<sup>2</sup>. Each variant included 4 repetitions, totalling an area of 28 m<sup>2</sup>.

A number of 5 experimental variants were established, as follows:

- V1, the untreated control;
- V2 treated with 52.5 g diatomite/repetition;
- V3 treated with 105.0 g diatomite/repetition;
- V4 treated with 210.0 g diatomite/repetition;
- V5 treated with *Trichoderma* T85-ICDPP collection, with radicular administration, respectively 2–3 granules per plant, in the planting phase.

In the 62<sup>th</sup> day of plant rise (May 2020), the solid diatomaceous earth was administered.

Specific technological protocols were performed, manual ploughing was performed (at the end of May), and at the beginning of June, the onion crop was sprayed with Fusilade/Galligan (1:1/ha).

In May-August 2020 period, 224  $\mbox{\rm I/m}^2$  of precipitation were registered.

The granulated NPK-S 20/20/0-13 chemical fertilizer complex was mechanically administered on gutters, by a tractor and bunker cultivator, for a period of 18 days (in June 2020), respectively 55–65 kg/1600m<sup>2</sup>. A diatomite suspension, 8.3:1, was administered after emergence for 119 days, by plant manual spraying using a Vermorel pump type (33% or 3.3 I of diatomite suspension per plant, respectively for V2, V3, V4 samples).

Periodically the treatments for pathogens agents and fungal infections were performed (Fusilade forte – Goal 4F, 1:0.5/ha).

128 days after emergence, the onions formed yellow shirts in 70% of the crop, about 60% leaves dried and lay about 60%.

The entire onion crop was harvested after 115 days of planting, respectively 170 days after emergence. The production was weighed on repetitions and variants. An average of 10 bulbs per variant for physicochemical studies and determinations, for both dehydrated leaves and fresh bulbs were selected.

For physico-chemical studies and determinations, ethanolic and aqueous extract onion bulbs and leaves were performed, according to Lee et al. [20] and Jiang et al. [21]. From onion leaf, water retention capacity and solubility index were performed according to Jiang et al. [21]; for aqueous and ethanolic onion leaf extracts, total polyphenol content, flavonoid content were performed according to Jiang et al. [21]. The parameters determined for the initial onion bulb product were pH and the dry matter; for total initial onion bulb extract, the essential parameters were total polyphenol content, total flavonoid [21], reducing sugar content and nitrogen content/ protein; for the ethanolic onion extract the determined parameters were also the total polyphenol and flavonoid content, following the same protocols.

## **RESULTS AND DISCUSSIONS**

Onions are grown for both green and dried consumption as bulbs. In the present study, the production of consumption bulbs was evaluated in different experimental variants (figure 1). The production of consumption bulbs was evaluated in different experimental treatments and it was observed that the sample V3 with 105 g of diatomite at 7 m<sup>2</sup> administered, has the highest production value recorded, respectively 148.53 kg/28 m<sup>2</sup>, compared to the untreated control V1, 123.99 kg/28 m<sup>2</sup> consumption bulbs.

Another variant of the study under observation was variant V5, where *Trichoderma* was administered. In this case, the bulbs production was superior to the control untreated sample V1, but inferior to the other variants, respectively 128.18 kg.

The production of onion bulbs of  $5.30 \text{ kg/m}^2$ , in the case of the V3 variant, treated with 105.0 g diatomite/ 7 m<sup>2</sup>, was comparable to other studies where an average production between 2.71–8.65 kg/m<sup>2</sup> was reported (as t/ha 27.1–86.5) [22].

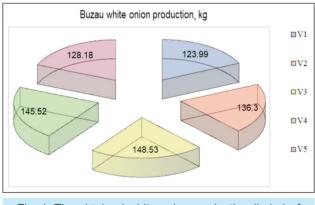


Fig. 1. The obtained white onion production (in kg) of consumption bulbs, in different experimental treatment, performed

The onion bulb pH value variation is presented in figure 2. The pH variation for the directly treated onion samples is essential, in order to establish the influence and product impact to applied treatment and also to study the experimental conditions product and productivity impact. It was observed that V1 control samples presented a lower pH value, above  $5.06 \pm 0.02$ , compared with the highest level over  $5.25 \pm 0.03$ , for V2 and V5 samples. Similar mean values of acidity ( $5.20 \pm 0.01$ ) were recorded for samples V3 and V4 samples.

It is known that onion has various very beneficial properties for our health, but a high acidity level can cause damage to the digestive system. The current trend in nutrition is to consume the most basic foods, a context in which the results obtained in our study can lead in addition to increasing onion productivity and agricultural products of superior nutritional quality. So, it was observed that sample V2 is the most recommended variant with average productivity.

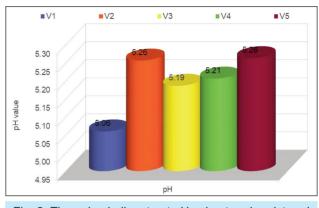
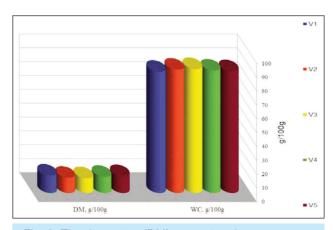
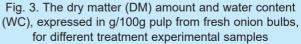


Fig. 2. The onion bulb extract pH value trend registered for all 5 culture types with differentiated treatment

Figure 3 presents the results obtained for fresh onion bulbs humidity studies by gravimetric assays. The fresh onion bulbs humidity analysis showed that the lowest diatomite concentration administrated to the V2 variant has a beneficial influence by high water content, a value compared to the highest level recorded for the sample treated with an almost double amount of diatomite (V3 samples).

The beneficial effect and impact on this sample are reflected and correlated with the lower acidity level determined. On the other hand, the amount of dry matter is the highest in control variant V1,  $12.55 \pm 0.1$  g/100 g, and the smallest in V3 samples, respectively 10.61 ± 0.35 g/100 g. By extrapolation, the studies performed showed the lowest humidity,  $87.45 \pm 0.1$  g/100 g, and the highest dry matter for the control untreated fresh onion bulbs, and the highest water content,  $89.39 \pm 0.3$  g/100 g and a small dry matter in 105 g diatomite/7 m<sup>2</sup> applied treatment for V3 fresh bulb products, according to figure 3. In the scientific literature with a specific profile, the dry matter values





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between 7.0–14.3 g/100 g fresh mass have been reported [22].

Total flavonoid contents of fresh and ethanolic extracts onion bulbs, obtained under different experimental conditions were performed [21].

The study and content analysis were performed both for the fresh onion bulb total extract and for ethanolic extract obtained, according to figure 4.

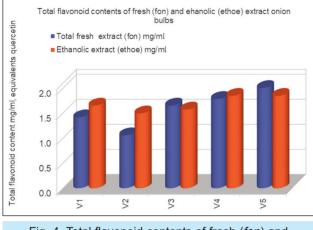


Fig. 4. Total flavonoid contents of fresh (*fon*) and ethanolic (*ethoe*) extract onion bulbs, obtained under different experimental conditions

The experimental cultivation conditions showed an important impact concerning the flavonoid content. Therefore, the determinations performed for the total extract from fresh products showed a variable flavonoids content, directly dependent on the applied diatomite treatment. The higher applied diatomite concentrations were conducted to higher flavonoid content. This data is correlated with the water content trend determined. There is a significantly low content of flavonoid for products where diatomite treatment was minimal (case of V2 samples with 52.5 g diatomite/7 m<sup>2</sup> treatment, 1.059 ± 0.023 mg/ml total flavonoid content).

The *Trichoderma* suspension treatment applied demonstrated their efficacy, both for average productivity and for the quality of a superior product. As consequence, the data obtained showed the highest levels of flavonoids for the various V5, respectively 2.005  $\pm$  0.076 mg/ml. Total flavonoid contents of ethanolic fresh bulb extract V4 and V5 samples were comparable, respectively 1.848  $\pm$  0.06 mg/ml, and the lowest values were found also for V2 (1.496  $\pm$  0.1 mg/ml), according to figure 4. The control V1 samples had a total flavonoid content at 1.653  $\pm$  0.06 mg/ml, being thus reported a higher value compared to V2 (1.496  $\pm$  0.1 mg/ml) and V3 onion samples (1.574  $\pm$  0.2 mg/ml) (figure 5).

The white onion peel powder was also subjected to total flavonoid content determination, in quercetin eq. (mg/ml). As consequence, the number of flavonoids was highest in V5 *Trichoderma* treated, respectively  $383.89 \pm 0.03$  mg/ml, followed closely by V4, 372.16  $\pm$  0.03 mg/ml flavonoid content reported. All experimental variants had flavonoids higher values found in

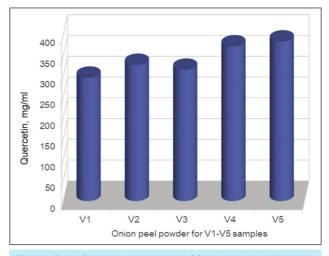


Fig. 5. Total flavonoid contents of fresh onion bulbs peel powder, obtained under different experimental conditions

onion peels, compared with the untreated control test V1 (296.69  $\pm$  0.007 mg/ml), according to figure 6. The polyphenols (in galic acid eq.) amount, extracted with ethanol, was found for the control V1 (0.834  $\pm$  0.01 mg/ml), followed by variant V5 (*Trichoderma* treated) with 0.723  $\pm$  0.009 mg/ml total flavonoid contents; the highest value was reported for V2, respectively 1.360  $\pm$  0.007 mg/ml, according to figure 7.

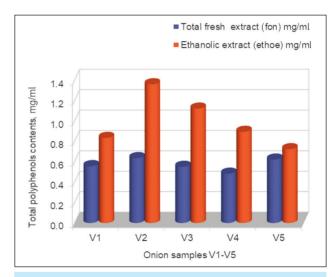
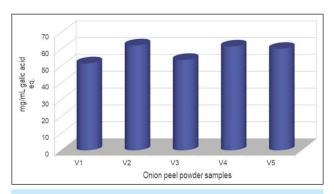
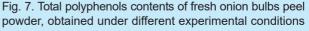


Fig. 6. Total polyphenols contents of fresh (*fon*) and ethanolic (*ethoe*) onion bulbs, obtained under different experimental conditions





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In total fresh extraction, variant V4 presented the lowest amount of polyphenols ( $0.483 \pm 0.005 \text{ mg/ml}$ ), control variant V1,  $0.557 \pm 0.01 \text{ mg/ml}$ , and variant V2 had the highest value, respectively  $0.635 \pm 0.005$ mg/ml, according to figure 6.

The number of polyphenols in onion peel powder, for products grown under different experimental conditions, the lowest level for control variant  $51.685 \pm 0.02$  mg/ml, and the highest for V2 ( $62.355 \pm 0.04$  mg/ml), were found, as shown in figure 7.

## CONCLUSIONS

The production of onion bulbs and their quality is strongly influenced by soil-climatic conditions and cultivation technology. Direct correlation with the number of nutrients administered, accompanied by additional water intake, under the conditions of predisposition to climate aridity and water-heat stress was found. It was reported a significant production increase for the V3 variant treated with 105 g diatomite/7 m<sup>2</sup>, compared to the control variant. At the same time, the control has the highest amount of dry matter, which increases the storage period. According to the results, the *Trichoderma* V5 sample had the highest flavonoids composition, both in the total and ethanolic fresh extract, followed by the variant V4 treated with 210 g diatomite/7 m<sup>2</sup>. Also, for the ethanolic flavonoid fresh extract, the highest amount was determined for V5 onion samples. The number of total polyphenols, in both variant V2 peels powder and the ethanolic extract, was the highest reported.

The research will be further developed by using agro textiles to protect crops from the action of extreme weather events, ensuring plant vigour and optimal conditions for pollination and fruiting, which can result in significant increases in production. Another experiment included tests on the behaviour of agro textile materials as a support for seed germination and soil mulching, in a conventional or ecological system [23].

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