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Chlorophyll Content and Leaf Morphological Features of *Capsicum annum* L. cv. 'Alexander' under Yeast and Caffeine Tested as Bio-fertilizers

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RESEARCH ARTICLE

Abstract

Bio-fertilizers are in the present more and more of interest as alternative nutritive inputs. This research aim was to test different doses of yeast and caffeine on pepper growth and chlorophyll content. The experiment in controlled conditions consisted of 8 phytocosms treatments with 3 yeast and caffeine doses 1%, 2%, and 3%, one mixed treatment (2%), and the control treatment in five replications. The yeast addition promoted all the tested morphological parameters especially the middle dose tested. Caffeine treatments induced a slow growth of pepper vegetative period, even a delayed development compared with the control treatment. In conclusion, the mixed treatment was the most efficient pepper bio-fertilizer and provided the highest values of leaf length, width, seedling height, and also total chlorophyll content.

Keywords: phytocosms, height, leaf length, leaf width, absolute chlorophyll units

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INTRODUCTION

Leaf chlorophyll is an important photosynthetic pigment (Jiang et. al., 2012). This indicator and pigment is reflecting plant health and photosynthesis capacity. Also, it can indicate a plant stress tolerance threshold (Taiz et al., 2015)) and nitrogen status (Chen et al., 2021). The nondestructive methods are commonly used for the purpose of estimating the leaf chlorophyll content (Ferrarezi et al., 2020). The use of chlorophyll meters represents an accurate method providing fast assessment (Schaper and Chacko, 1991) of the entire plant system growth under different conditions (Jiang et al., 2004). The extraction methods of pepper leaf chlorophyll were significantly correlated with different chlorophyll meter readings; therefore, the last mentioned it became a reliable tool for determining this parameter (Madeira et al., 2003; Madeira and Varennes, 2005; Ling et al., 2011; Schaper and Chacko, 1991). Sweet pepper, an annual plant from *Solanaceae* Family, is one of the most popular and consumed vegetables worldwide (ALKahtani et al., 2020). Because of its rich antioxidant content, red sweet pepper was intensively studied, highlighting the high content of vitamin C (Deepa et al., 2006; Ibrahim et al., 2019; Papathanasiou et al., 2021). Pepper fruits represent a true reservoir of several different vitamins and phenolic compounds (Papathanasiou et al., 2021). When

the growth was improved and leaf morphological characteristics increased because of the inputs addition, the leaf chlorophyll content increased (ALKahtani et al., 2020; Madeira and Varennes, 2005; Pereira et al., 2021). Even if it was found that bio-fertilizers liquid or solid could improve plant growth, morphological and physiochemical parameters (Padilla et al., 2018), it was also found a higher chlorophyll content in the absence of any bio-fertilizer (Pereira et al., 2021). It was proved that the physiological parameter respectively the chlorophyll content is sensitive to additional nitrogen compounds (Padilla et al., 2018), hence it is not applicable to all plant species. The bio-fertilizer application could provide plant seedlings intensive growth, speed in development, and vigourousness. Coffee grounds it is usually applied to several ornamental plants as bio-fertilizer. Together with yeast, caffeine represents cheap bio-fertilizers that could improve plant performance (Ruta and Farcasanu, 2020; Kocaefe-Özşen et al., 2022). To test the effect of simple and combined yeast and caffeine as bio-fertilizers, it was set the aim of assessing the chlorophyll content and some morphophysiological paramenters as growth drivers respectively plant height, leaf width and length.

MATERIALS AND METHODS

Experimental design and plant material

The experiment was conducted under controlled conditions, 40% humidity, 20±°C to test pepper growth and leaf chlorophyll content under yeast and caffeine different doses. A number of eight treatments were tested with 3 yeast doses, D1, D2, and D3%, caffeine in 3 doses C1, C2, and C3%, one mixed treatment (MIX) with the middle dose of yeast: caffeine D2:C2%, and a control treatment (M) in five repetitions (Figure 1). All 40 phytocosms were randomized and kept at a photoperiod of 13 h light and 11 h dark at natural light intensity for 2 months.

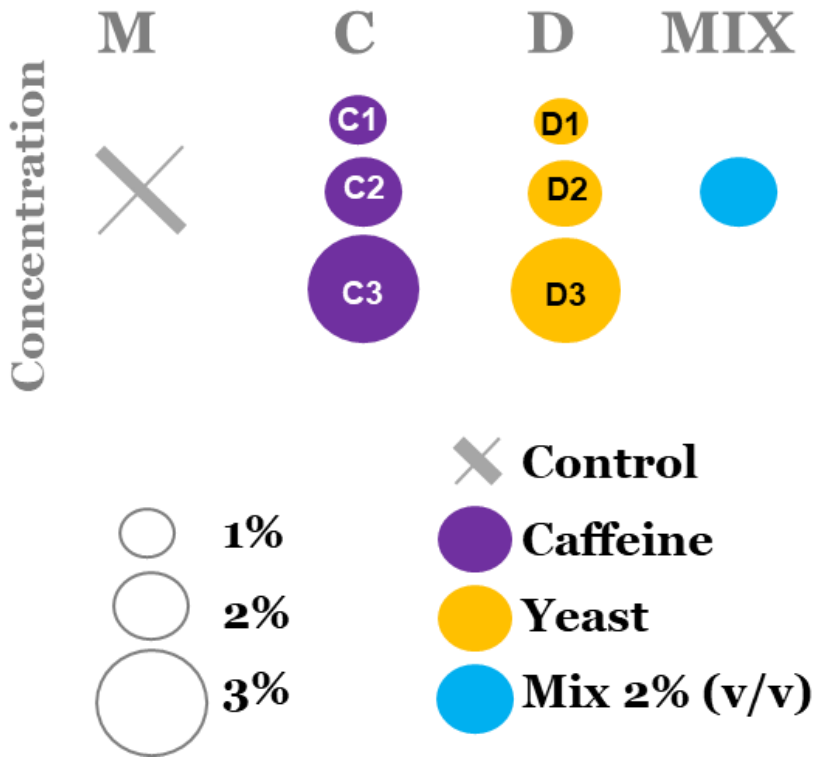

Figure 1. Experimental design with schematic tested treatments

Seeds of pepper *Capsicum annum* cv. Alexander were taken into study. A number of 10 seeds were sown in each container, 5 on one side, and 5 on the other side. All the seeds were sterilized in a concentration of 50% (v/v) distilled water and ethyl alcohol, then stirred with circular moves for 10 minutes and rinsed 5 times with 100 ml distilled water (Lindsey et al., 2017).

Soil properties

The phytocosms consisted of plastic containers filled with 100 g soil with humidity in the range of 60–70%. At the beginning of the experiment, the tested concentrations were prepared in 10 ml water and later the water was added by spraying on the soil phytocosms. The humidity was kept constant by weighing during the experiment. Soil

pH was between 6.5–7 and organic carbon value of 13.96. The Florisol soil (producer: S.C. FLORISOL PRODUCT S.R.L.) was purchased from the market. The physico-chemical characteristics were in this case previously analysed and reported as percentages per kg. The nitrogen content was 1.78%, phosphorus value was 0.21% and potassium content was 0.82%. The organic matter content was 34.48%.

Measurements and data analyses

In the experiment, the plants were monitored on a twice-weekly basis to record the parameters. The rapid assessment of chlorophyll content as leaf greenness was done using a SPAD chlorophyll meter MC-100 S/N Apogee Instruments. This represents a non-destructive method and it is often used because it accurately displays the chlorophyll concentration without damaging the plant material. The chlorophyll meter was calibrated before use, and the amount of chlorophyll is expressed in absolute units μmol m^{-2} of chlorophyll. It measures a round area of 9 mm in diameter.

The chlorophyll content of sweet pepper measured by a spectrophotometer provided a relationship between the SPAD values and the total chlorophyll content (Madeira et al. 2003). The conversion of the SPAD values into total chlorophyll followed the equation:

$$\text{Total chlorophyll } (\mu g\ cm^{-2}) = 1.182 \times SPAD + 16.158$$

The Caliper method was used for determining leaf length and width using an electronic micro-meter Micron Tools. All the seedling heights were measured with a ruler. The soil was weighted with a precision scale Kern EHA (0.01). Statistical analyses were performed using RStudio Software (Stoian et al., 2022; Vâtcă et al., 2022). The difference between mean values was determined according to the post-hoc LSD multiple comparison test.

RESULTS AND DISCUSSIONS

Obtained results regarding the leaf lengths of the pepper seedlings, significant differences were observed depending on the applied treatment (Table 1). In general, all treatments with yeast recorded higher values compared to the control, and those with caffeine had the opposite effect. The pepper seedlings were grown in the MIX treatment highlighted a significant difference of 27.33mm higher than the control. The mixed treatment recorded significant leaf length values compared to the first and the lowest dose of yeast applied. This proves that an average dose combined between caffeine and yeast provides a favourable nutritional environment for the growth and development of pepper plants. In this case, the negative effects of caffeine were counteracted by the addition of yeast (Mekki and Ahmed, 2005). The leaf length of the pepper seedlings in the mixed treatment is also significant compared to all caffeine treatments tested. The values of the leaf's length in the treatments with caffeine showed negative values compared to the control. It is interesting that the dose of caffeine C2 produced plants with an average leaf's length with 3.01mm smaller than the control and the first lower dose tested C1 caused a bigger difference of 0.50mm in the pepper leaves length. The highest dose of C3 caffeine applied helped the seedlings to develop a leaf length very close (−1.34mm) to those from the control. These differences, although statistically not significant, support the theory of some pathways activation by caffeine that positively influences the growth and development of the seedlings (Ruta and Farcasanu, 2020). Between the different doses applied within the same treatment, there are no significant differences strictly related to the leaf length of the pepper plants. However, the width is influenced in the case of applying different dosages of yeast. The average dose D2 determined significant differences compared to the first dose applied D1 in the case of the leaf's width parameter. This finding also explains the superiority offered by the mixed treatment compared to all the assessed parameters (Madeira and Varennes, 2005). The same tendency of decreasing width values of the pepper leaves was observed when caffeine was applied. The lowest difference (2.23mm) compared to the control was observed when applying the average dose of 2% caffeine C2 (Table 1).

Table 1. The length (L) and width (l) of the pepper leaf. Results are presented as average ± SE and the values between brackets are the differences compared with the control treatment

Treatment	L (mm)	l (mm)
M	15.67±0.33 c	8.40±0.1 c
C1	12.16±1.01 (−3.51) c	5.47±0.43 (−2.93) c
C2	12.66±1.20 (−3.01) c	6.17±0.78 (−2.23) c
C3	14.33±1.20 (−1.34) c	5.87±0.29 (−2.53) c
D1	32.66±1.45 (+16.99) b	18.66±1.20 (+10.26) b

D2	38.33±3.17 (+22.66) ab	22.66±0.33 (+14.26) a
D3	35.66±4.70 (+19.99) ab	21.66±2.18 (+13.26) ab
MIX	43.00±1.73 (+27.33) a	25.33±1.76 (+16.93) a

Different letters represent $p < 0.05$; between brackets, it could be observed the difference from the control treatments (M)

Plant height at the end of the experiment registered the highest values (91.00±9.53 mm) in the MIX treatment significantly higher by 64 mm compared to the control (Figure 2). The treatments with yeast independent of different doses tested produced significant seedling height compared with the caffeine treatments. The highest seedling height from the yeast treatments was in D2 for 87.00±4.35 mm with 60 mm more compared to the control, followed by the first tested dose of 78.00±7.02 mm. The lowest seedling height was in D3 (66.66±10.10 mm) with the highest yeast dose tested. Also, the seedlings decreased in height with increasing the caffeine doses. The lower seedling height from all the tested treatments was obtained in the caffeine treatments C3 (−5.00 mm) and C2 (−3.67 mm), with not a significant decrease compared with the control treatment (Figure 2).

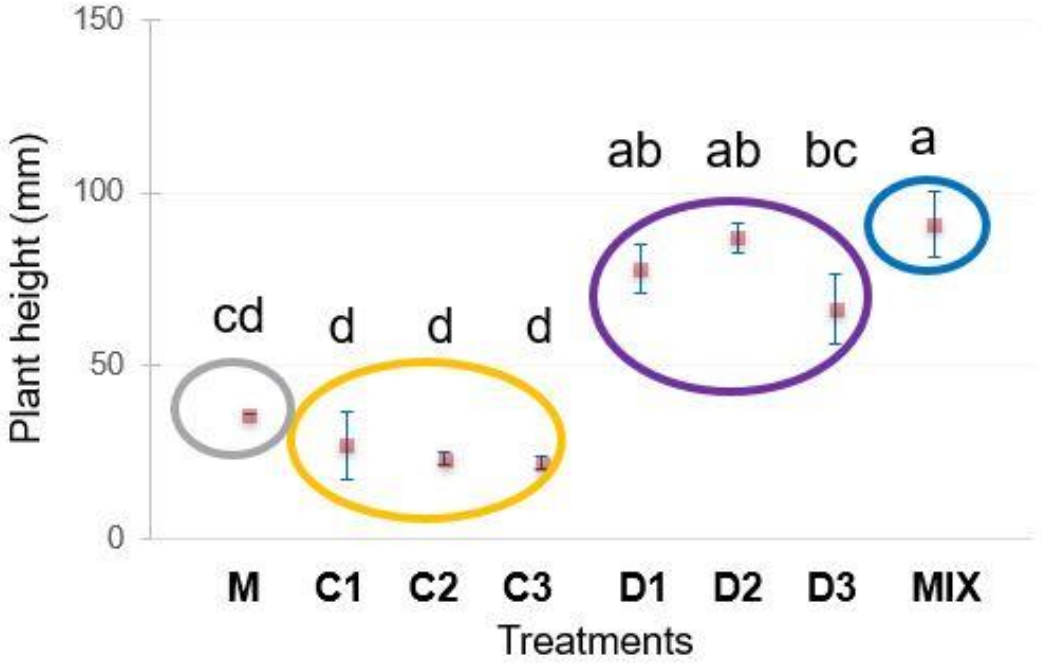

Figure 2. Pepper seedlings height (mm) of all treatments; M-control; C1-caffeine 1%, C2-caffeine 2%, C3-caffeine 3%, D1-yeast 1%, D2-yeast 2%, D3-yeast 3%, MIX-caffeine:yeast (v/v) 2:2%. Different letters post-hoc LSD highlight significant differences at $p < 0.05$

The total chlorophyll content followed the same tendency as seedling height with the respect to higher differences between the treatments with yeast compared with the control and caffeine (Figure 3). In terms of total chlorophyll content, the highest value was registered in the MIX treatments of 408.4±13.2 μg cm^{-2}, significantly higher compared will all tested treatments except D2. All yeast doses produced significantly higher leaf chlorophyll content compared with the control. The highest value of leaf total chlorophyll from the yeast tested doses was in D2 with 337.89 μg cm^{-2} higher than the control, followed by D1 with 314.87 μg cm^{-2} higher compared with the control. Indeed, as all these treatments registered leaves with higher length and width had also superior values of chlorophyll content. Other studies found similar results in pepper leaf chlorophyll content which increased with leaf length development (Ku et al., 2010). This particular aspect was observed visually during the experimental trial (Figure 4). The control treatment had the lowest total chlorophyll content of 49.73±3.18 μg cm^{-2}. This result was also seen in other studies that linked the chlorophyll content with nitrogen addition (Padilla et al., 2018, Ruta and Farcasanu, 2020; Kocaefe-Özşen et al., 2022).

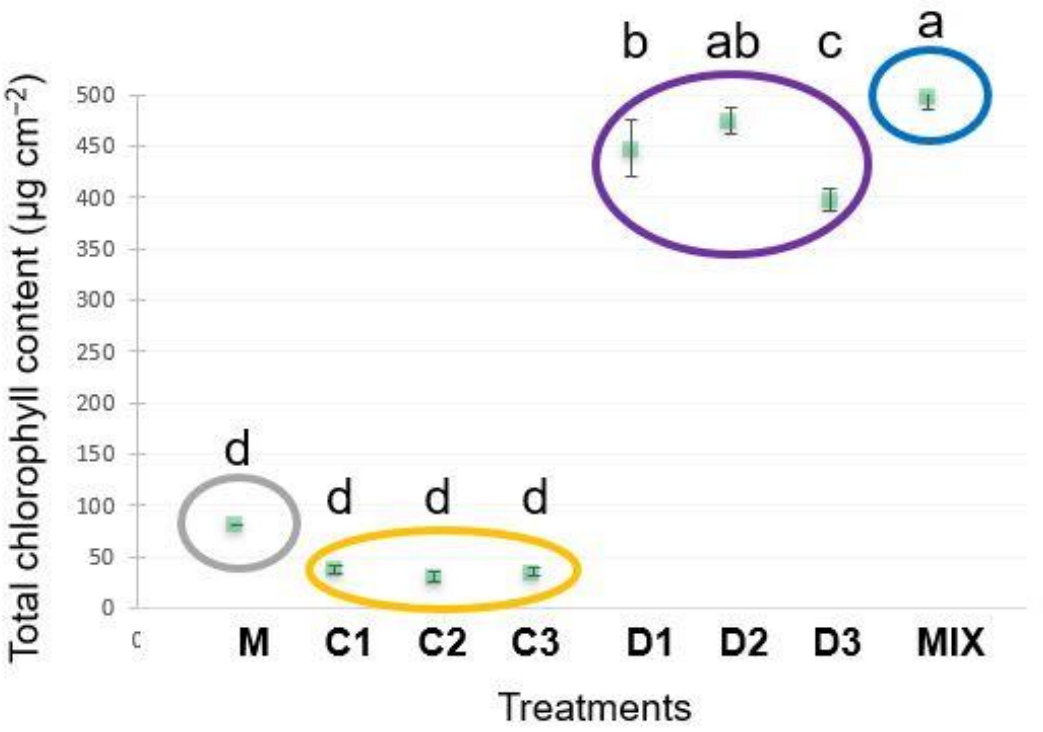

Figure 3. Total chlorophyll content (µg cm^{-2}) from leaves of all treatments; M-control; C1-caffeine 1%, C2-caffeine 2%, C3-caffeine 3%, D1-yeast 1%, D2-yeast 2%, D3-yeast 3%, MIX-caffeine:yeast (v/v) 2:2%. Different letters post-hoc LSD highlight significant differences at $p < 0.05$

Figure 4. Pepper seedlings growth and development under all treatments tested

CONCLUSIONS

The total chlorophyll content is directly influenced by the leaf morphological characteristics length and width and also by the height of the seedling. The treatment with both yeast and caffeine v/v 2% was the most effective in terms of all tested parameters. Yeast addition doubled the values of leaf lengths and width compared to the control. Seedlings' height was three times higher in the yeast treatments. Total chlorophyll content increased 8 times because of yeast addition compared to the control. Overall parameters the yeast doze of 2% gave the highest values. Caffeine addition decreased the leaf length and width between a range of 1—4 mm. At a 3% caffeine dose, the leaf length was higher compared with the two other doses. At a 2% caffeine dose, the leaf width was higher compared

with the other two tested doses of caffeine. As the caffeine doses increased, the seedlings' height decreases. The total chlorophyll content was the highest at the first tested dose and the lowest at the second tested dose.

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