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Biostimulators Impact Germination and Leaf Development of *Capsicum annum* cv. Alexander – a BBCH Scale Assessment

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Abstract: Yeast and coffee grounds were tested as biostimulators on pepper growth. The main aim was to emphasize the growth differences when these two biological agents were added. A number of 40 microcosmos experiments were set up with yeast (D1-3) and coffee ground (C1-3) in three concentrations 1, 2, and 3%, a mixed treatment (MIX) with the middle concentration (2%) and a control (C) treatment in five repetitions. The growth and development were reported on BBCH scale. The yeast (D1) produced 48% of the seedlings in 14 BBCH, D2 produced 16% seedlings in 18 BBCH. The coffee grounds C1 (11 BBCH) and C2 (12 BBCH) produced 12% fewer seedlings than the control treatment. Seedlings from the MIX treatment were at the end very similar with D3 treatment with the higher achievement of 35% in 14 BBCH. Pepper seedlings' growth and development were easily visible between different treatments tested.

Keywords: coffee grounds, growth, pepper, yeast.

Introduction

Many studies have been conducted to reveal the mechanisms of action of caffeine in the presence of *Saccharomyces cerevisiae* as a model eukaryotic cell driver (Ruta and Farcasanu, 2020; Hu et al., 2016; Kocaefe-Özşen et al., 2022). A model organism is used in scientific research for various reasons: simplifying the biological context (Mohammadi et al., 2015), overcoming ethical and experimental constraints (Gresham et al., 2008), eliminating redundancies (Chen et al., 2012), establishing a framework for the development and optimization of analytical methods. It was emphasized that a model

organism should be representative of a larger class of living organisms (Karathia et al., 2011).

S. cerevisiae, a relatively simple unicellular eukaryote, has emerged as a versatile and robust model organism to study the fundamental factors that determine eukaryotic cell biology (Duina et al., 2014). Yeast addition provided insights into the complex mechanisms underlying sensitivity and response to external conditions, including exposure to a multitude of natural and synthetic chemicals such as caffeine. *S. cerevisiae* is generally sensitive to caffeine, as this substance has been found to affect yeast cell growth and morphology, DNA repair mechanisms, intracellular calcium homeostasis, and cell cycle progression (Kuranda et al., 2006).

Also, in *S. cerevisiae*, it has been observed that sensitivity to caffeine can be correlated with defects in the cell wall integrity pathway and that caffeine activates the signaling when cell wall stability can be monitored in response to osmotic or heat stress (Levin, 2005).

Up to this point, knowledge is mainly at the empirical level. Ornamental plants passionate in particular, know and agree with the beneficial effects of using coffee grounds and yeast in optimal quantities for the good development of plants in general.

The aim of this paper was to highlight the pepper germination capacity and leaf development percentages of different secondary stages when yeast and coffee grounds were applied.

Materials and methods

The experimental design consisted of 8 treatments with yeast (1%, 2%, and 3%), coffee grounds in the same concentration as yeast, a mixed treatment (2%), and a control treatment only with soil in five replications (Figure 1). The pepper seeds (*Capsicum annum* cv. Alexander) were sterilized with an absolute ethyl alcohol reagent for analysis (C_2H_5OH). All the seeds were sterilized by placing them 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. A number of 10 seeds were sown in each container, 5 on one side, and 5 on the other side. Water application was carried out by spraying, with approximately 25 ml of warm water daily in the first part of the day to maintain optimal soil moisture for germination.

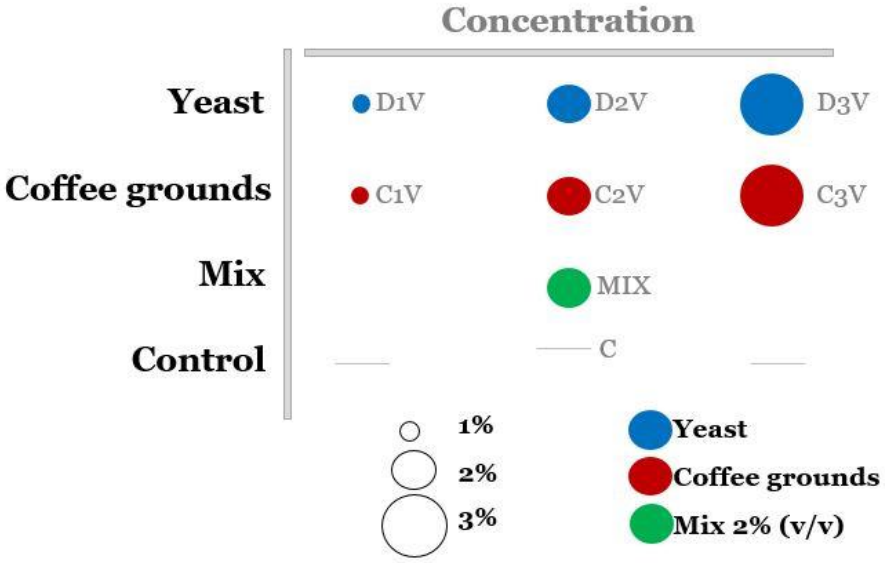

Figure 1. Experimental design

A number of 40 microcosms were set up in total. The experimental containers were made from plastic filled with 100g soil. The soil amount weighing was carried out using the Kern EHA 500-1 (0.01). A commercial product (Florisol produced by S.C. FLORISOL PRODUCT S.R.L.) suitable for plant growth and development was used as a substrate in the experiment (Table 1).

Table 1

The experimental soil physico-chemical characteristics

No. crt.	Parameters	Values
1.	Organic dry matter	Min 70%
2.	pH	6.5-7.0
3.	Humidity	60-70%
4.	Nitrogen (N)	1.78%
5.	Phosphorus (P)	0.21%
6.	Potassium (K)	0.82%
7.	MO	34.48%
8.	Organic carbon	13.96

The treatments were placed at room temperature 20±2°C with a fluctuating photoperiod respectively 15 days with 12h light and 12h dark, 30 days with 13 × 11, and 15 days with 14 × 10.

The method used for assessing pepper growth was BBCH scale

(Biologische Bundesanstalt, Bundessortenamt CHmical). It identifies phenologically differences in the growth and developmental stages of pepper and it represents the newest and uniform coding system of plants' phenological growth stages (Stoian et al., 2022). The treatments were daily assessed and the values were registered when differences were observed.

The germination capacity (Catană et al., 2020) was calculated by the formula:

$$GC = NG / TN \times 100$$

NG=total number of germinated seeds
TN=total number of tested seeds

Results and discussion

The control treatment registered the highest germination capacity of pepper of 88%. With 8% less germination capacity was observed in the MIX treatment compared to the control. Yeast produced a germination capacity in the range of 60-68% with the lowest value at the lowest tested dose and the highest value at the middle dose. The coffee grounds treatment has a higher range when it comes of germination capacity, between 53-76% following the same pattern as the yeast treatments (Figure 2).

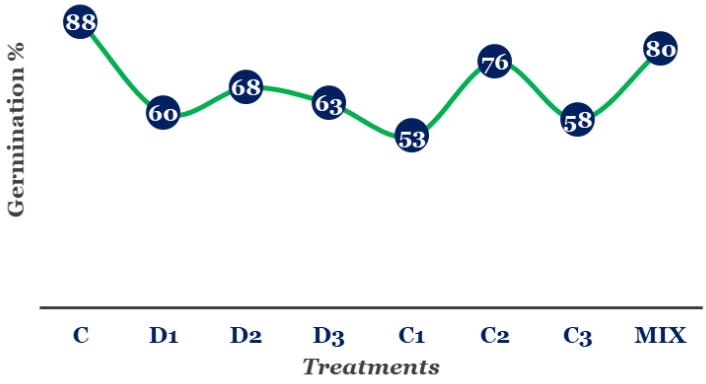

Figure 2. Germination capacity of pepper due to the tested treatments (C-control; D1-yeast 1%; D2-yeast 2%; D3-yeast 3%; C1-coffee grounds 1%; C2-coffee grounds 2%; C3-coffee grounds 3%; MIX-yeast:coffee grounds 2:2%)

In the following tables, the lowest values were presented with blue color and the highest values were presented with red color.

The germination principal growth stage according to Meier (2018) ended with 09 BBCH. In this secondary stage, pepper succeeded to emerge at the soil surface. The present study recorded as a supplementary secondary stage 09+BBCH, where the seedling had folded cotyledons.

Pepper seedlings from the control treatment needed at least 21 days to obtain more than 50% with completely unfolded cotyledons in the 10 BBCH (Table 2). The next secondary stage with the first true leaf visible 11BBCH was achieved by more than 50% on day 56. At the end of the experiment, 6% of the seedlings from the control treatment were in 10 BBCH, 60% were in 11 BBCH and 22% were in 12 BBCH with two true leaves completely unfolded (Table 2).

Table 2

Pepper plants growth on the BBCH scale for each secondary stage percentage values in the control treatment

No.	Day	BBCH	07	09	09+	10	11	12	13	14	15	16	17	18
1	12			38										
2	15			26	16									
3	16			34	8	28								
4	19			30	10	37								
5	21			12	6	60								
6	22			11	7	62								
7	23			16	4	62								
8	27					65	20							
9	28					49	38							
10	56					6	60	22						

The seedlings from the coffee grounds treatment developed differently depending on the different doses tested (Table 3). The first dose produced after 12 days with 20% seedlings (09 BBCH) less compared with the control treatment. Then after 3 days, the seedlings in 10 BBCH were with 18% higher than the control. On day 16 the most advanced stage of the seedlings 10BBCH was 2% lower than the control. In the middle of the experiment time (day 21) the seedlings from 09 BBCH has the same percent (12%) in the C1 and control treatments. The next secondary stage 10 BBCH was represented by a lower percent (42) compared with the control (Table 3). In the next

two days, the seedlings in 10 BBCH almost doubled their number, however, this number was 27% lower compared with the control. On days 27 and 28 the 10 BBCH was represented by the seedlings with 38 respectively 22% lower than the control. On the same days, the 11 BBCH was 4% higher than the control and 14% lower than the control. At the last assessment, the seedlings in 11 BBCH were 12% lower than the control.

The coffee ground dose 2% (C2) produced a number of seedlings in 09 BBCH higer by 14% than the control. Only in day 19, the secondary stage 10 BBCH was doubled in number compared with C1 (1% dose) and with 2% lower than the control treatment. In day 22 it was achieved 50% threshold of the seedlings in 10 BBCH with 12% lower than the control. At the last assessment, the 2% dose produced 66% of the seedlings in 11 BBCH. This number was higher by 18% than the first applied dose and with 6% higher that the control. The seedlings in the next secondary stage 12 BBCH were with 12% lower than the control.

The C3 coffee grounds dose of 3% had the germination (09 BBCH) very similar to the control treatment. After 15 days, there were already 18% seedlings in 10 BBCH and none in the control. The percentages are more evenly distributed for each secondary stages between the time range of 16-13 days. All these percentages were in general lower compared with the control treatment. The higher caffeine doze applied produced seedlings in 12 BBCH, with 12% more than in the control and with 36% lower for the 11 BBCH (Table 3).

At the end of the experiment, all the treatments with different coffee grounds doses had a total seedlings percentage lower than the control.

The treatments with yeast determined an evenly seedling growth, and development compared with coffee grounds treatments (Table 4). The first two doses (1% and 2%) had a germination capacity (09 BBCH) lower by 2% compared with the control. The third dose D3 determined seedlings with 4% higher than the control. The yeast D1 produced seedlings in 11 BBCH on day 27 with 36% higher than the control. At the last assessment, it was observed the most higher percentage (48) in 14 BBCH and only 2% of the seedlings were in 17 BBCH.

Table 3

Pepper plants growth on BBCH scale for each secondary stages percentage values in the coffee grounds treatments (C1-1%, C2-2%, C3-3%)

No.	Day	BBCH	07	09	09+	10	11	12	13	14	15	16	17	18
C1														
1	12		6	18										
2	15			11	12	18								
3	16		2	7	8	26								
4	19			24	4	16								
5	21			12	16	18								
6	22			12	2	32								
7	23			6	10	35								
8	27					27	24							
9	28					27	24							
10	56					5	48							
C2														
1	12			52										
2	15			20	35	5								
3	16			42	3	20								
4	19			32	2	35								
5	21			18	14	38								
6	22			10	8	50								
7	23			4	10	59								
8	27					55	18							
9	28					37	38							
10	56						66	10						
C3														
1	12			39										
2	15			17	8	18								
3	16			38	4	22								
4	19			28	1	14								
5	21			16	10	20								
6	22			14	12	23								
7	23			12	8	30								
8	27					28	24							
9	28					22	30							
10	56						24	34						

Yeast dose 2% (D2) produced >50% of the seedlings in 10 BBCH after 22 days, with 11% less that the control treatment (Table 4). On day 27, 50%of the seedlings were in 11 BBCH for both treatments doses 2 and 3%, a value with 30% higher that the control. At the last assessment, the yeast influence D2 produced 12% of the seedlings in 14 BBCH and 16% in 18 BBCH.

The higher yeast dose D3 registered a low seedlings percentage until day 23 compared with the previously tested doses. Days 27 and 28 produced similar seedlings' development as D1 in the treatments with 3% yeast (d3). At the end of the experiment, the higher yeast dose tested produced higher seedlings percentages around 34% in 14 BBCH lower (14%) compared with D1 and higher (10%) compared with D2 (Table 4).

The assessment after 12 days for the MIX treatment is missing because no difference was observed compared with the other treatments (Table 5). However, after 15 days an important difference was noticed, >50% of the seedlings had already completely unfolded cotyledons 10 BBCH. The following days 21-23 registered a range of 1-4% seedling development increase compared to the control.

Also on day 22, the MIX treatment overcomes the control with 3% for 11 BBCH. On day 27, the MIX treatments jumped one secondary stage with more than 60% in 11 BBCH compared to the control. At the end of the experiment, 35% of the seedlings were in 14 BBCH very similar to D3 treatment. Also, there were 17% of seedlings in 15 BBCH and 3% in 16 BBCH (Table 5).

Table 4

Pepper plants growth on BBCH scale for each secondary stages percentage values in the yeast treatments (D1-1%, D2-2%, D3-3%)

No.	Day	BBCH	07	09	09+	10	11	12	13	14	15	16	17	18
D1														
1	12			36										
2	15			6	8	27								
3	16			4	10	30								
4	19			6		40								
5	21			6	6	35								
6	22			12		38								
7	23			10		48								
8	27					2	56							
9	28					5	54							

10	56									48	10		2		
D2															
1	12		36												
2	15		16	15	24										
3	16		16	14	25										
4	19		10	16	30										
5	21		6	12	41										
6	22		6		51	2									
7	23		6	4	50	2									
8	27				13	50									
9	28				16	48									
10	56					4	12	12	24						16
D3															
1	12		42												
2	15		22	22	20										
3	16		28	4	13										
4	19		26	8	15										
5	21		22	8	21										
6	22		20	4	28										
7	23		14	6	34										
8	27				4	50									
9	28				5	56									
10	56					11	4	12	34	2					

Table 5

Pepper plants growth on BBCH scale for each secondary stages percentage values in the MIX treatments (2% (v/v))

No.	Day	BBCH	07	09	09+	10	11	12	13	14	15	16	17	18
1	15			13	3	53								
2	16			23	8	40								
3	19			6	5	63								
4	21			7	10	60								
5	22			3	5	66	3							
6	23			3		63	8							
7	27					15	63							
8	28					4	75							
9	56								25	35	17	3		

All these values were very easily visually observed (Figure 3).

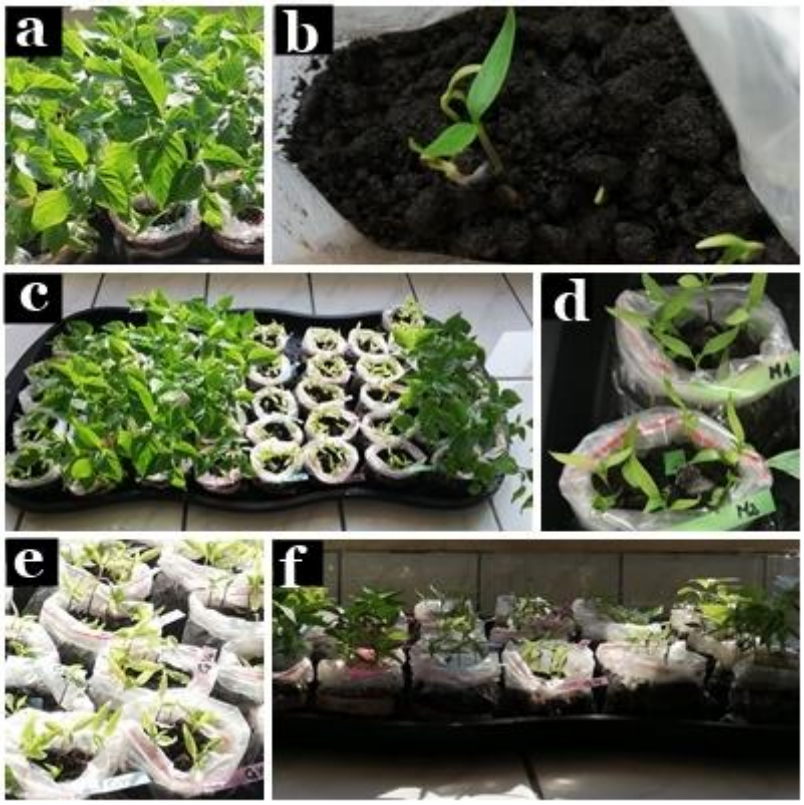

Figure 3.Pepper grown in yeast treatments (a); Seedlings with folded 09+ BBCH and unfolded cotyledons 10 BBCH (b), General experimental aspects (c, f), Control treatments (d), Coffee ground treatments (e).

Yeast is most widely used by the research community due to its capability for genetic studies, comprehensive genome annotation (Goffeau et al., 1996), and a high degree of homology of essential cellular organization and metabolism with higher eukaryotes (Castrillo et al., 2004).

In addition, *S. cerevisiae* is an invaluable tool in genomic studies, resistance profiling, metabolome studies, and metabolic engineering (Matuo et al., 2012; Dos Santos et al., 2015; Lian et al., 2018; Nielsen, 2019; Coronas-Serna et al., 2020).

Assessment of the interaction between caffeine and yeast cells have demonstrated the existence of additional caffeine targets, including components of cell wall integrity pathways (Moser et al., 2000). The cell wall of *S. cerevisiae* confers cell shape and protection

against harsh environments (Cid et al., 1995). It consists of different types of molecules, including nanoproteins, glucans and chitin, closely interconnected.

Conclusions

- The pepper growth phenophases and time of the first true leaves appearance were different depending on the treatment.
- The highest germination capacity was observed in the control treatments followed by the MIX treatment.
- The most vigorous seedlings were seen in the mixed and yeast treatments.
- In the treatments with yeast (10%), it was observed the highest germination capacity and the first seedling first the first true unfolded leaf. In this treatment was assessed a plant with 8 leaves in the 08 BBCH stage. On average, the plants in yeast treatments were in the 05 BBCH stage.
- During the experiment, especially at the beginning, all seedlings had about the same growth rate, but towards the end, the coffee grounds treatments stagnated, stopped growing, and started to turn yellow. On average, the plants in the coffee grounds treatment were in 02 BBCH the same as in the control treatments.
- In the end, the results from the MIX treatment were by far the most interesting and unexpected. Coffee grounds inhibit plant growth physiological processes and yeast, on the contrary, accentuates and promotes them. Altogether, both biostimulators provided only advantages by counteracting any inhibitory effects on seedling growth.

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