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EFFECTS OF BIOLOGICAL AND CHEMICAL TREATMENTS ON THE MORPHOLOGY AND PRODUCTIVE PERFORMANCE OF SOME *Camelina sativa* L. VARIETIES

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Abstract. *Camelina sativa* is an oleaginous plant of economic perspective, with major importance in the production of biofuels. In addition, camelina oil has several nutritional benefits and valuable uses in many industrial branches. The aim of this study was to evaluate the effect of biological and chemical seed treatments on six varieties of camelina. The morphological and technological characteristics of seed treated and untreated plants were evaluated during vegetation up to full maturity. Positive results were recorded at the camelina genotypes G1- Mădălina, G3-GP 204, G4-GP 202 and G6-Camelia when seeds were treated with plant beneficial bacteria. Therefore, biologically seed treated plants expressed an increased biomass weight /plant (g), seed weight/plant (g) and a higher percentage of seed weight from the total biomass weight (%).

Keywords: *Camelina sativa*, seed production, controlled conditions.

Rezumat. Efectul unor tratamente biologice şi chimice asupra caracterelor morfologice şi performanţelor productive ale unor soiuri de *Camelina sativa* L. Această specie reprezintă una dintre plantele oleaginoase de perspectivă şi cu importanţă majoră în obţinerea biocombustibililor. Pe lângă aceasta, ea are multiple recomandări nutriţionale şi de valorificare în multe ramuri industriale. Obiectivul acestui studiu a fost evaluarea efectului a două tratamente (biologic şi chimic) aplicate la seminţele a şase genotipuri de camelina. În cadrul studiului au fost evaluate caracterele morfologice şi proprietăţile tehnologice ale plantelor tratate la sămânţă şi netratate, până când acestea au atins maturitatea deplină. Rezultate pozitive au fost înregistrate la genotipurile de *Camelina sativa* G1-Madalina, G3-GP 204, G4-GP 202 şi G6-Camelia când seminţele au fost tratate cu bacterii benefice. Astfel, seminţele plantelor tratate biologic au evidenţiat o creştere a parametrilor de productivitate analizaţi: greutate biomasă/plantă (g), greutate seminţe/plantă (g) şi procentul de seminţe raportat la greutatea totală a biomasei (%).

Cuvinte cheie: *Camelina sativa*, producerea de seminţe, condiţii controlate.

INTRODUCTION

Camelina sativa (L.) Crantz, popularly known as false flax, wild flax, Siberian oilseed, German sesame or gold-of-pleasure, is an ancient cultivated oilseed species (ZUBR, 1998) that occurs mostly as accompanying flax. It is an annual or winter annual plant that belongs to *Brassicaceae* family (*Cruciferae*).

Its history goes back to the Bronze Age (PUTNAM et al., 1993*)*. Although widely cultivated up to the early 1940's, the commercial production ceased with the introduction of oilseed rape. The lower cost of hydrogenating rape oil and the lack of knowledge on the value of oils containing a high percentage of polyunsaturated fatty acids were the main causes of the lack of interest in *C.-sativa* (BUDIN et al., 1995; SHUKLA, 2002; JOHNSON et al., 2010; DOBRE et al., 2014a,b). The revival of interest in camelina oil now is due to its high linolenic acid content (up to 38%) (ZUBR, 1992). Linolenic acid is one of the OMEGA-3 fatty acids, which are only found in linseed and edible fish oils (ZUBR, 1997; CROWLEY & FRÖHLICH, 1998; DRUMEA et al., 2016).

C. sativa (L.) Crantz, has favourable agronomic traits and potentially large number of uses. It has a short growing season (PAVLISTA et al., 2011) and can be cultivated as an annual summer crop or as annual winter crop. It has good potential for non-irrigated crop production systems (DOBRE et al., 2014b), the seasonal water consumption ranging from 333 to 423 mm (FRENCH et al., 2009). It is a cold and drought tolerant crop (MATEI et al., 2014). The chemical fertilizers requirement is moderate to low (100 kg/ha of N; 30 kg/ha of P; and 40 kg/ha of S) (DOBRE et al., 2014a). Minimal management practices are required after seeding to crop maturity (TONCEA, 2014). Camelina can be grown in environmentally friendly way with oat, without excessive applications of herbicides and pesticides, due to its allelopathic effects against weeds and its high resistance to blackleg disease of cruciferous plants (PUTNAM et al., 1997). It can be grown on marginal land as a low input biofuel crop, except for heavy clay and organic soils. This specie is self–pollinating and the seeds do not have seed dormancy. It has negligible potential as an invasive weed if grown on a large-scale as a biofuel crop (SHONNARD et al., 2010; FRÖHLICH & RICE, 2005). Camelina oil could offer excellent health benefits to consumers and has nutritional value to humans and animals (DRUMEA et al., 2016). The oil is rich in tocopherols, which confers a good oxidative stability and a reasonable shelf life. Some co-products like glycerin and camelina meal are used in animal diet (PETRE et al., 2015; GIURESCU et al., 2016). The oil is also used in biodiesel production (via transesterification) and for renewable jet fuel (through hydrogenation/hydrocracking) (TONCEA, 2013); it can be also used to obtain biobased industrial products such as: polymers, varnishes, paints, cosmetics and dermatological products, and after epoxidation could be used for lubricants, resins, coatings, adhesives, soaps etc. Moreover, it is compatible with the existing agricultural and fuel infrastructure (WALSH et al., 2012; VOLLMANN & LAIMER, 2013). The life-cycle assessment (LCA) showed a reduction of C emissions by 75-80% when using camelina bio-fuel (PETRE et al., 2015).

Recent interest for the use of renewable sources of raw materials for biofuel production requires the identification of effective solutions to pursue the principles of sustainable development. This approach is the one that meets the needs of the present without compromising the ability of future generations to meet their own requirements.

Numerous studies have been carried out to maximize seeds performance at sowing, in order to control plant pests and diseases through biological and chemical means (CONSTANTINESCU & SICUIA, 2013; SICUIA et al., 2015).

It has been noticed that, in field conditions, camelina is frequently affected by some diseases, the most frequent being the downy mildew produced by *Peronospora parasitica* (VOLLMANN et al., 2001). In the spring, the oospores of the pathogen germinate and cause the primary infection, from which conidia are spread on the host plants. After the first symptoms of disease, the pathogen attack can proliferate on the leaves, stems and fruits (silicules) of camelina due to more conidia production, which causes secondary infections (SPENCER, 1981). In Romania, the first report of downy mildew on camelina was made by CRISTEA & MANOLE (2014) on a Romanian variety cultivated at "Moara Domnească" farm of the University of Agricultural Sciences and Veterinary Medicine of Bucharest.

Moreover, the incidence of phytopathogenic infections caused by *Phytium* spp. and *Phytophtora* spp. is increasing in greenhouse conditions, as it also happens at legume and ornamental plants.

The purpose of this study was to evaluate some morphological traits and the productive performance of six camelina varieties cultivated in greenhouse, when using some biological and chemical seed treatments, based on *Bacillus amyloliquefaciens* and Dithane M 45 fungicide. The experimental study was carried out in automated controlled greenhouse conditions.

MATERIAL AND METHODS

The experiment was developed in the greenhouse automation unit for research of the Center for Quality Research of the Agro-Food Products (HORTINVEST), from UASVM-Bucharest. The camelina plants were grown in pots, on 160 m^2 greenhouse with heating, shading, air-conditioning, assimilation lighting, tide type irrigation system and microaspersion facilities. The provenance of the biological material was assured from - NARDI Fundulea Romania for the seeds of camelina genotypes.

Six varieties of *Camelina sativa,* were used in this study: G1- Mădălina hybrid, (Romanian genotype, registered in 2014); G2- Fundulea local population of wild camelina, (Romanian genotype); G3-GP 204 (Spanish genotype); G4-GP 202; G5-Calena (Austrian genotype); G6-Camelia variety, (Romanian genotype, registered in 2011).

As a working method we have resumed in this experiment the conditions of camelina cultivation in protected areas according to the protocol developed and reported in an earlier publication (PODGOREANU et al., 2015).

Camelina was seeded in black plastic, square-shaped pots of 11x11x11 cm in size, placed in water collecting trays of 60 x 40cm. Each pot was filled with 1L of Kekkila DSM 2 W growth substrate, which is a light, aerated peat (well milled) with additional perlite, pre-fertilized with NPK 14-16-18, with the pH adjusted to 5.5/5.9. Before use, the growth substrate was sterilized by autoclaving at 100° C for 20 minutes, and soaked in water after cooling.

For the present study, the trials were conceived as a bifactorial experiment, where the **A Factor** involved two experimental seed treatments compared to an untreated control:

- "M" = Untreated control, where seeds were moistened for 20 minutes in distilled water;
- "B" = Biological treated experimental variant, where seeds were immersed for 20 minutes in bacterial suspension based on *Bacillus amyloliquefaciens* BW strain, at 10^8UFC/ml concentration, supplemented with 2% carboxymethyl cellulose;
- "D" = Chemical treated experimental variant, where seeds were immersed for 20 minutes in 0.2% solution of Dithane M 45, a contact fungicide (80% mancozeb).

The **B Factor** involved the morphological characteristics and productive parameters were recorded in the six Camelina genotypes: **G1**-Madalina, **G2**-Fundulea local population of wild camelina, **G3**-GP 204, **G4**-GP 202, **G5**-Calena and **G6**-Camelia.

The bacterial bio-preparation was obtained at the Biotechnology department of UASVM Bucharest. Liquid bacterial culture of 48h was used to prepare the inoculum.

The *Bacillus amyloliquefaciens* BW strain was grown in Luria Bertani broth at 28°C in a rotary shaker, at 150 rpm. The bacterial culture was centrifuged for 15 minutes at 3500xg and the pellet was washed and resuspended in phosphate saline buffer. To increase the bacterial adherence to seeds tegument during inoculation (as seed treatment) the aqueous suspension was amended with 2% carboxymethyl cellulose. The bacterial cell concentration in the formulated product was 10^8UFC/ml.

The biological treatment based on *B. amyloliquefaciens* BW strain was selected to be used due to the beneficial properties of the strain. The BW strain expressed activity both *in vitro* and *in vivo* against a wide spectrum of plant pathogens and improved plant growth of several leguminous species (CORNEA et al., 2008; SICUIA, 2013).

Dithane M45 is a contact fungicide with a wide spectrum of pathogen control in field crops, potato, vegetables, flowers, orchards and vineyards, as well as seed treatment. Due to its multisided mode of action, it prevents pathogen resistance. The active substance mancozeb 80%, inhibits spore germination and interrupts six enzymatic pathways.

Ten seeds of camelina from each genotype were sowed /pot under two replications in each experimental variant and the plants were examined during the 5 months of culture - from February (the 2nd decade) to July (the 1st decade) (Table 1).

Table 1. Experimental scheme used to evaluate performance on six camelina genotypes cultivated in the HORTINVEST greenhouse unit (spring, 2016).

GENOTYPE (HYBRID)	Number of plants/ Variant "M"		Number of plants / Variant "B"		Number of plants / Variant "D"		Total evaluated plant/ genotype
	Repetition MR1	Repetition MR2	Repetition BR1	Repetition BR2	Repetition DR1	Repetition DR2	
G1 - Mădălina	**10**	**10**	**10**	**10**	**10**	**10**	**60**
G2-Fundulea local population of wild camelina	**10**	**10**	**10**	**10**	**10**	**10**	**60**
G3-GP 204	**10**	**10**	**10**	**10**	**10**	**10**	**60**
G4-GP 202	**10**	**10**	**10**	**10**	**10**	**10**	**60**
G5-Calena	**10**	**10**	**10**	**10**	**10**	**10**	**60**
G6-Camelia	**10**	**10**	**10**	**10**	**10**	**10**	**60**

Legend: "M" = Untreated control, "B"= Experimental variant of biocontrol treatment based on plant beneficial bacteria, "D" = Experimental variant with chemical control treatment based on Dithane M 45 contact fungicide.

The following morphological characteristics and productive parameters were recorded during the bifactorial experiments: plant height (cm); it was measured periodically during vegetation and at harvest time from the soil surface to the highest point of the plant; days to flowering; it was measured periodically during the number of days from date of seeding to approximately 20%, 40%, 60% and 80% of the plants having open flowers; days to maturity; it was estimated visually as the date when approximately 90% of pods were brown; biomass weight (g); seed weight/plant (g); the difference between seed weight/total biomass (%).

RESULTS AND DISCUSSIONS

The greenhouse experiment conducted in the spring of 2016, at HORTINVEST research centre, Bucharest, revealed some morphological and productivity performances in six genotypes of *Camelina sativa* plants, with or without chemical or biological seed treatments.

The growth conditions registered during camelina cultivation were as follows: 4700 to 6000 lx naturally provided 14 hours/day. However, during cloudy periods, the lighting was supplemented with four halogen lamps placed on each corner of the growning area. The temperature was maintained between 24 and 40°C during daylight and at 20°C during night, while the relative humidity was adjusted between 55 and 80% (Fig. 1).

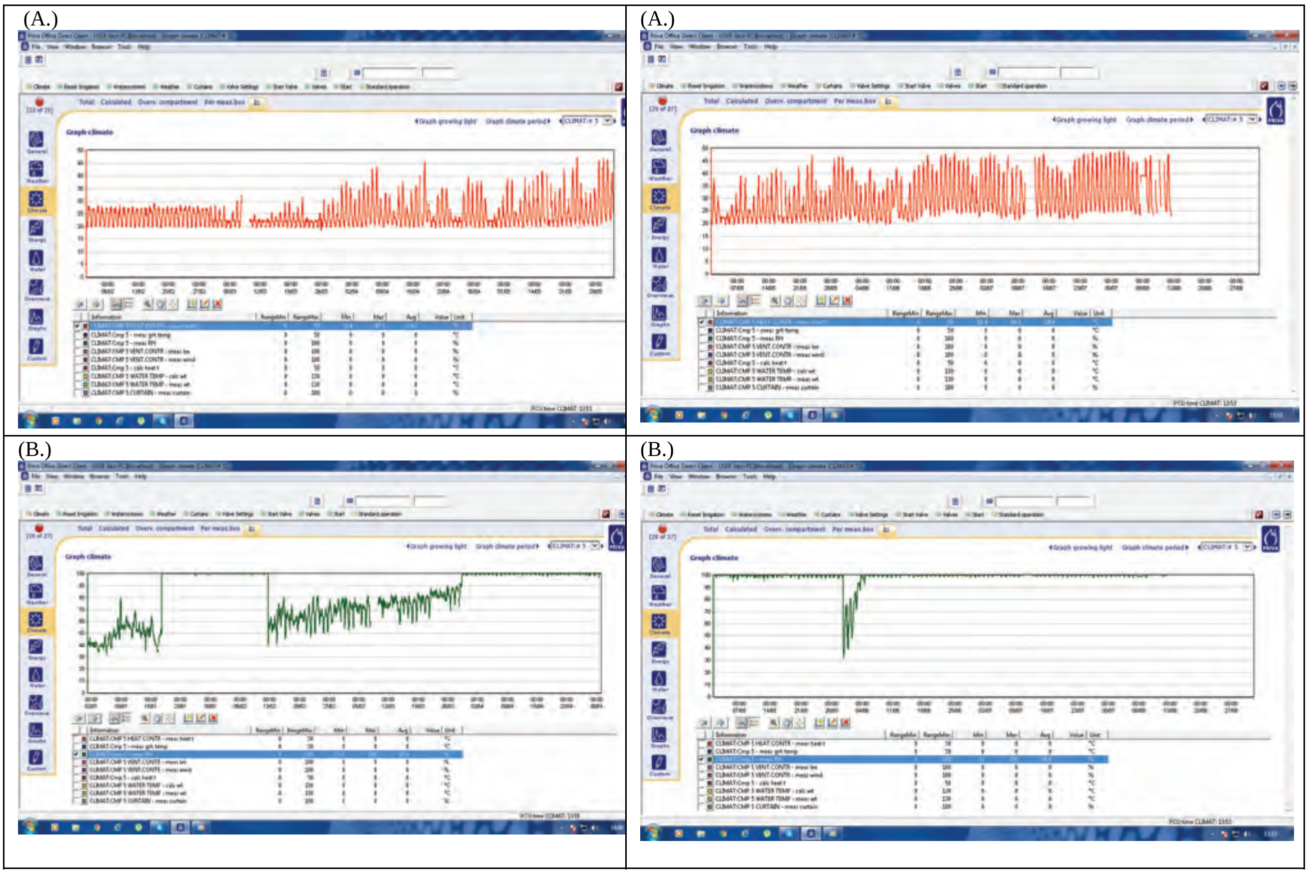


Figure 1. Registered values for Temperature - T°C (A) and Atmospheric humidity-U% (B) in the HORTINVEST greenhouse unit during of camelina genotypes studies (spring, 2016). (Original photo-in the HORTINVEST greenhouse unit, spring 2016).

In order to prevent the pest attack, yellow sticky traps were used, and curatively, a 0.5% of ORTHUS solution was applied 2-3 times, each 7-day. The germination of the seeds of all six genotypes of camelina, treated and untreated, was analyzed at 3 to 10 days after sowing. The germination rate after the first 3 days from sowing was relatively moderate.

In all replicates of the treated experimental variants (BR1, BR2 and DR1, DR2) the germination rate was 55%, compared to the control (MR1, MR2). For all six genotypes (G1 to G6), full germination (100%) was recorded after 8 days in the biological treated variant "B" and the control "M", and after 10 days in the chemical treated variant "D". All germinated camelina plants showed normal germline characteristics (Fig. 2).

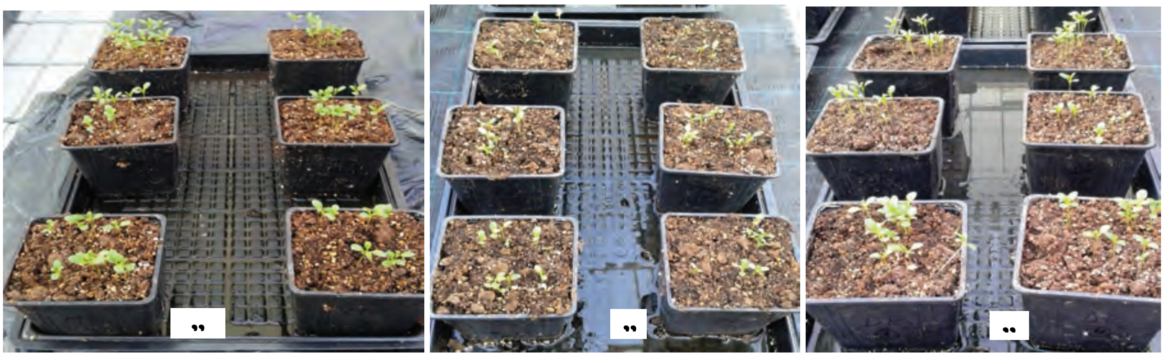


Figure 2. Germination of camelina plants 10 days after sowing. "M" = Untreated control, "B"= Experimental variant of biocontrol treatment based on plant beneficial bacteria, "D" = Experimental variant with chemical control treatment based on Dithane M 45 contact fungicide. (Original photo-in the HORTINVEST greenhouse unit, spring 2016).

To determine the dynamics of plant growth (cm) for all six camelina genotypes (G1-G6) in the control and treated variants, three measurements were performed: after 35 days from sowing, and at 20 days intervals (Table 2). In the first growth stages, plants height at 35-day and 55-day after sowing was higher in the control variants than in the biologically and chemically treated variants (Fig. 3).

Table 2. Morphometric determinations of plant height (cm) for six camelina genotypes (G1-G6) cultivated in greenhouse conditions, in three experimental variants. (Average values/ plants/2 repetition).

GENOTYPE	**Variant "M"**			**Variant "B"**			**Variant "D"**		
	Average of plants height (cm)								
VARIANTS	after **35** days	after **55** days	after **105** days	after **35** days	after **55** days	after **105** days	after **35** days	after **55** days	after **105** days
G1-Mădălina	24.5	32.5	54.0	15,5	22.5	50.5	21.0	26.0	48.0
G2-Fundulea local population of wild camelina	22.5	30.5	60.0	19.0	26.0	59.0	21.5	27.0	57.0
G3-GP 204	13.5	27.5	52.5	17.0	22.5	**60.0**	15.0	22.5	46.0
G4-GP 202	25.5	32.8	55.5	14.5	19.6	**58.0**	21.0	29.0	47.0
G5-Calena	24.5	31.5	56.0	15.0	20.0	**57.0**	18.5	30.0	53.0
G6-Camelia	19.5	29.0	57.0	13.5	18.5	**61.0**	11.0	28.0	51.0

Legend: "M" = Untreated control, "B"= Experimental variant of biocontrol treatment based on plant beneficial bacteria, "D" = Experimental variant with chemical control treatment based on Dithane M 45 contact fungicide.

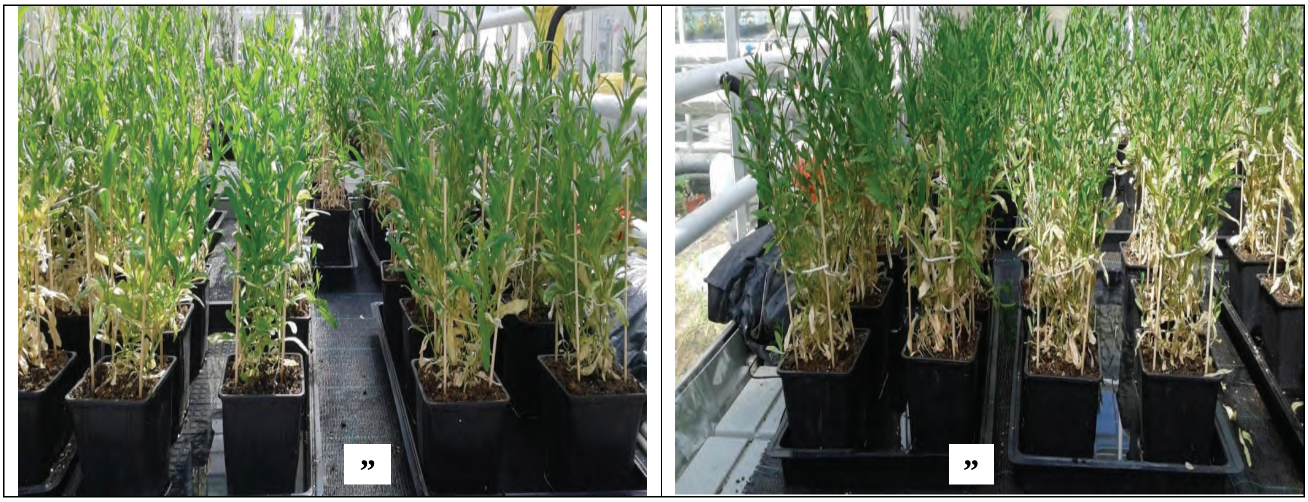


Figure 3. Camelina seedlings aspect of experimental variants (M, B, D), after 55 days from sowing: "M" = Untreated control, "B"= Experimental variant of biocontrol seed treatment based on plant beneficial bacteria, "D" = Experimental variant with chemical seed control treatment based on Dithane M 45 contact fungicide. (Original photo-in the HORTINVEST greenhouse unit, spring 2016).

However, as the camelina plants approached maturity, plants vigour improved. Morphometric analyzes revealed that plants height increased 10 to 20 cm/30 days and the number of leaves increased to 30-40 leaves/plant.

Thus, at the 3rd determination of plans height (after 105 day from sowing), in four camelina genotypes: **G3**-GP 204, **G4**-GP202, **G5**-Calena and **G6**-Camelia, biologically seed treated with *B. amyloliquefaciens* BW strain, it was found that the height of the plants exceeded with 7-14% the untreated control. The emergence of the first flower buds was revealed after 40 days from sowing, and extended for another 20 to 40 days. The flowers blossomed at first on the main stem of the plants and then on the lateral branches (Table 3).

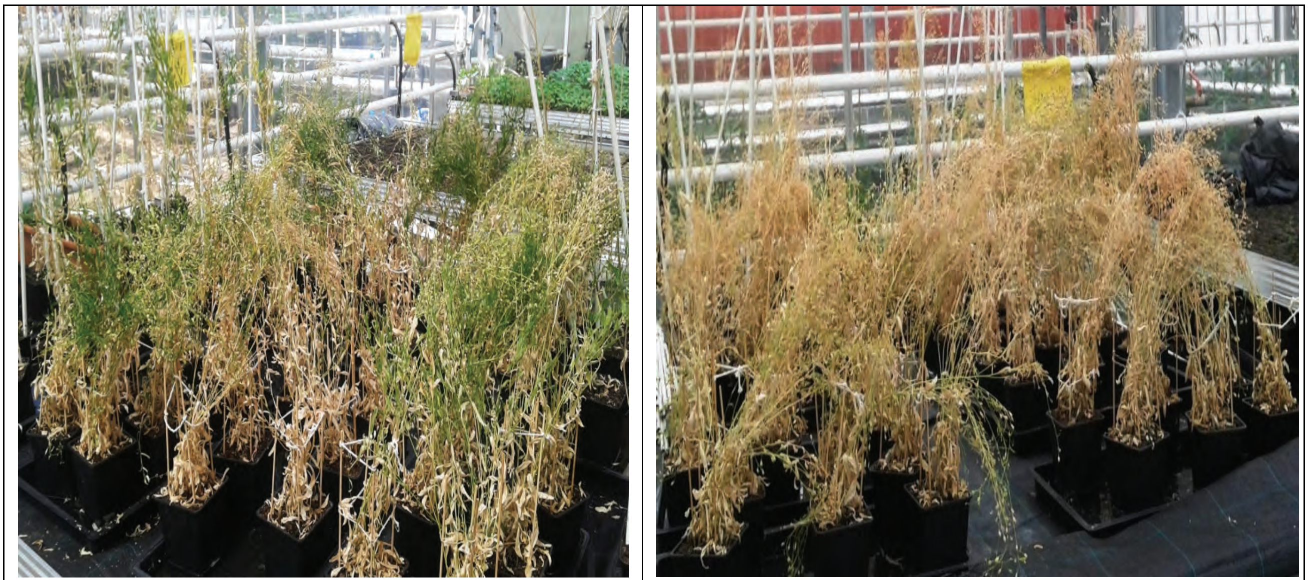
Table 3. Time estimation of vegetative and generative stages, in no. of Days until flowering and no. of Days until maturity, for six camelina genotypes (G1-G6) cultivated in greenhouse conditions, in three experimental variants. (Average values/ plants/2 repetition).

GENOTYPE	**Variant "M"**		**Variant"B"**		**Variant"D"**	
VARIANTS	Days to flowering	Days to maturity	Days to flowering	Days to maturity	Days to flowering	Days to maturity
G1 - Mădălina	62	108	68	120	72	115
G2 - Fundulea local population of wild camelina	63	105	66	115	70	126
G3-GP 204	63	125	64	115	77	126
G4-GP 202	62	105	76	125	69	121
G5-Calena	66	115	64	115	72	126
G6-Camelia	68	108	80	133	72	121

Legend: "M" = Untreated control, "B"= Experimental variant of biocontrol treatment based on plant beneficial bacteria, "D" = Experimental variant with chemical control treatment based on Dithane M 45 contact fungicide.

No significant differences were found between the genotypes regarding the time period needed for plants to bloom and mature their silicules in the untreated/control variants. The only differences were noticed at the blooming of G6-Camelia genotype, where the plants biologically treated (variant "B") blossomed 12-18 days after the first flowers appeared in the control ("M"), and 3-11 days after the plants chemically treated (variant "D"). The generative stage, from the time of the first fructification (silicules) to full maturity, another 40 days were required, for all six studied genotypes, in both control "M" and "D" experimental variants. Similar results were also registered in "B" experimental variant (biologically treated), however at **G1** – Mădălina genotype, form the end of blooming to silicules maturity plants staggered over 50 days.

The *C. sativa* plants from all three experimental variants, "M", "B" and "D", at the beginning of the maturing of silicules (80 days old plants) and prior to the harvest time (plants aged 120-125 days) are illustrated in Fig. 4.



a.) b.)

Figure 4. Camelina plants from all three experimental variants ("M", "B", "D"):
a) after 80 days and b) after 120 days from sowing (original photo-in the HORTINVEST greenhouse unit, 2016).

Plants productivity of all six camelina genotypes (G1- Mădălina, G2- Fundulea local population of wild camelina, G3-GP 204, G4- GP 202, G5-Calena and G6-Camelia) cultivated in greenhouse condition, in seed treated and untreated experimental variants were evaluated after harvest and presented in Table 4.

Table 4. Plants productivity parameters of six camelina genotypes (G1-G6) cultivated in greenhouse conditions in seed treated and untreated experimental variants. (Average values/ plants/2 repetition).

GENOTYPE / VARIANTS	Variant "M"			Variant "B"			Variant "D"		
	Biomass weight (g)	Seed weight/ plant (g)	Total seed weight/ biomass %	Biomass weight (g)	Seed weight/ plant (g)	Total seed weight/ biomass %	Biomass weight (g)	Seed weight/ plant (g)	Total seed weight/ biomass %
G1-Madalina,	10.15	0.76	7.48	**17.55**	**1.13**	6.44	12.64	0.54	4.27
G2-Fundulea local population of wild camelina	16.30	0.91	5.58	14.63	0.76	5.19	12.44	1.53	12.29
G3-GP 204	11.23	0.45	4.00	14.36	1.24	8.63	11.46	0.24	2.09
G4-GP 202	14.78	0.94	6.35	**17.20**	**2.20**	**12.80**	14.66	0.70	4.77
G5-Calena	12.65	2.58	20.39	14.36	1.30	9.05	12.25	0.44	3.60
G6-Camelia	14.64	1.12	7.65	**17.52**	**2.32**	**13.24**	11.78	0.56	4.75

Legend: "M" = Untreated control, "B"= Experimental variant of biocontrol treatment based on plant beneficial bacteria, "D" = Experimental variant with chemical control treatment based on Dithane M 45 contact fungicide.

Analyzing plants productivity (table 4), it was found that the biological seed treatment of *Camelina sativa* genotypes G1 - Mădălina, G3-GP 204, G4-GP 202 and G6-Camelia increased the productivity potential of the plants, compared to the untreated control (M), regarding: biomass weight / plant (g), seed weight / plant (g) and the difference between weight of seeds and total biomass weight.

CONCLUSIONS

This experiment was carried out in 2016, during 5 months. Six *Camelina sativa* genotypes were analyzed for their morphometric and productivity potential, after being seed treated with biological and chemical products, compared with untreated controls. All plants were grown in automatic greenhouse, in the same climatic conditions and agronomic maintenance. The chemical seed treatment with Dithane M 45 contact fungicide provided higher morphometric characteristics like: plant height and number of shoots / plant. However, after harvest, the biological seed treatment with *Bacillus amyloliquefaciens* BW beneficial bacteria improved plants productivity of four genotypes (G1-Mădălina, G3-GP 204, G4-GP 202 and G6-Camelia) compared with the control and chemically treated variants.

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