

STIMULATING EFFECT OF ROMAGROBIOFERTIL NP FERTILISER ON TOMATO, CUCUMBER AND CAULIFLOWER CROPS

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Abstract

ROMAGROBIOFERTIL NP is a biological fertiliser on the basis of three bacterias belong to Azospirillum lipoferum, Azotobacter chroococcum and Bacillus megaterium species. Experiments were done during 2016 using tomatoes (Prekos F1), cucumbers (Mirabelle F1) and cauliflower (Cornell F1). The product was applied both in the seedling phase and after planting. Two treatments were applied on seedlings: 4 days before sowing on the surface of the peat in normal cell trays and 15 days after emergence. During vegetation period were performed three applications, using 15 l/ha in the following moments: first application, immediately before planting (in same day) on soil surface, the second, as a foliar spraying after recovery of the plants, and the third, 30 days later. In tomato, cucumber and cauliflower seedlings the application of Romagrobiofertil NP has positively influenced the plant height (> with 5.6 -17.9%), the number of leaves / plant (> with 20.0-29.6%), the number of roots /plant (> with 19.5 – 40.8%) and the length of the roots (> with 21.2 -33.1%). In tomato, cucumber and cauliflower crops, the effect of the product has been reflected both in the growth and development of plants and in the obtained yield higher with 2.3% on tomatoes, 3.7% on cucumbers and 2.4% on cauliflower. Additional obtained yield was significant in comparison with untreated control variant, in all three vegetable crops.

Key words: bio fertilizer, *Azospirillum lipoferum*, *Azotobacter chroococcum*, *Bacillus megaterium*

INTRODUCTION

Evolution of modern agriculture has led to extensive use of agricultural chemicals which have degraded the soil fertility, soil profile and rendered the soil unsuitable for plant growth. Bio fertilizers are being recognized as an essential component for increasing the sustainable agricultural productivity and maintaining long term soil fertility in an eco-friendly manner [1].

Under certain environmental and soil conditions, *Azospirillum* can positively influence plant growth, crop yields and N-content of the plant. This plant stimulatory effect exerted by *Azospirillum* has been attributed to several mechanisms, including biological nitrogen fixation and auxin production [2]. The mode of action of *Azospirillum* is most probably composed of multiple mechanism. The increased use of the various biological process in soil will decisively contribute to make agriculture more productive with less harm to the environment [3].

The isolation of phosphate solubilizing bacterial strains exhibiting high ability to solubilize soil phosphorus is a matter of great interest with high applicability. The use of phosphate solubilizing bacteria as inoculants simultaneously increase phosphate uptake by the plant and increase crop yield. Strains from the genera *Pseudomonas*, *Bacillus* and *Rhizobium* species are the most powerful phosphate solubilises [4].

MATERIAL AND METHOD

Experiences were carried out in 2016 on tomato crops, Prekos F1 and Mirabelle F1 cucumbers in protected areas and Cornell F1 cauliflower in the field.

The product was applied both in the seedling phase and after planting. Two treatments were applied on seedlings: 4 days before sowing on the surface of the peat in normal cell trays and 15 days after emergence.

During period of crops vegetation, there were applied three applications with Romagrobiofertil NP using the rate of 15 l / ha, in 500 l of water at the following times: the first treatment before planting (in the same day) by spraying the solution to the soil surface, the second foliar spray after recovering of the plants and third treatment after 30 days.

Experimental scheme: 2 variants - the control and treatment with Romagrobiofertil NP, in 4 replicates, the block randomized method, the plot area 15 m².

Phenological and morphological observations:

- tomato protected crops: apparition of the first flower in the first inflorescence, the first fruit to the size characteristic of the variety, the ripening of 10% of the fruits, the ripening of 80% of the fruits;
- cucumber protected crops: the appearance of the first flower, the apparition of the first fruit;
- cauliflower field: the beginning of inflorescence formation, inflorescence maturation.

Romagrobiofertil NP is a biological fertilizer based on bacterial cultures belonging to *Azospirillum lipoferum*, *Azotobacter chroococcum* and *Bacillus megaterium*.

The product contains a mixture in equal parts of the following bacterial cultures:

- *Azospirillum lipoferum*, strain Beijerinck 1925 from the Belgian collection BCCM/LMG, minimum 1x10⁹UFC/ml
- *Azotobacter chroococcum*, strain Beijerinck 1901 from the Belgian collection BCCM/LMG, minimum 1x10⁹ UFC/ml
- *Bacillus megaterium*, strain NCIMB 8508 from the British collection NCIMB, minimum 1x10⁹ UFC/ml.

Azospirillum lipoferum metabolizes a wide variety of organic materials in the soil (cellulose, hemicellulose, lignin, xylose), which multiply and colonize plant roots by associatively fixing atmospheric nitrogen.

Azotobacter chroococcum fixes free nitrogen from the atmosphere based on existing energy resources in the soil, metabolizes phosphates, assimilates and harnesses root exudates, counteracting pathogenic microorganisms in the soil.

Both bacteria provide nitrogen, growth factors and phytohormones, with biostimulating action on all crop plants.

The stimulant effect is not only a consequence of nitrogen fixation but also of the synthesis of antibacterial, antifungal and growth stimulators (auxins, giberellins, cytokines, siderophores and phytohormones).

Bacillus megaterium decomposes detritus, transforms insoluble phosphates from the soil into soluble forms, easily assimilable by plants. Provides phosphorus and organic acids (lactic, glutamic, succinic, propionic) and enzymes, with biostimulating action on plants.

Through the synergistic action of the three bacterial components on the decomposition of phosphates and inorganic nitrogen in the soil, repeated administration of the product contributes to the recovery of the soil by cleaning it with chemical fertilizers and obtaining a BIO culture.

Romagrobiofertil NP is administered in a rate of 15 l / ha aqueous solution (5 litres of *Azospirillum lipoferum* + 5 liters of *Azotobacter chroococcum* + 5 liters of *Bacillus megaterium*) in 500 liters of water.

The three components of the product are mechanically or manually mixed in 500 liters of water and administered by spraying with back pack sprayers in small plantations or spraying machines in large plantations.

Climate data was recorded throughout the vegetation period (Table 1). The highest temperatures were recorded during July 31-August 2nd, when several weather warnings were issued by NIMH Bucharest, for the south-east, including Bucharest. On July 31, the temperature exceeded 37 °C (yellow heat Code), August 1st temperature exceeded 39 °C (orange heat code) and on August 2 over 37 °C (yellow heat code).

The summer of 2016 was poor in precipitation(except August), in June it was 30.5 mm, in July only 2 mm, August 106.5 mm (of which 17 mm on August 12, 17.5 mm on 18 August and 58 mm on August 23) and in September 33 mm. The multiannual average rainfall in the Vidra area is 77 mm in June, 64 mm in July, 58 mm in August, and 42 mm in September. The rainfall between June and September was 68 mm compared to the multiannual average.

Table 1. Climatic data (Vidra, 2016)

Period	Temperature (°C)			Atmospheric humidity (%)			Rain (mm)	Soil temperature at 8 a.m.
	lowest	highest	average	lowest	highest	average		
Greenhouse climatic data for seedlings								
February 20-29	4.1	24.7	11.6	64.6	97.9	64.6	-	7.4
March 01-10	6.5	29.9	14.7	56.2	97.9	82.5	-	9.1
March 11-20	3.3	26.5	11.8	54.2	96.5	79.8	-	10.2
March 21-31	4.6	29.3	14.8	55.1	97.4	79.9	-	12.7
April 01-10	8.8	40.1	22.1	35.4	92.9	68.3	-	15.7
April 11-20	13.5	38.1	22.6	43.6	93.6	72.1	-	18.2
April 21-31	8.1	33.2	18.8	40.4	94.1	70.4	-	22.0
Greenhouse climatic data for tomatoes and cucumber cultures								
August 01-10	18.5	41.2	29.1	30.6	92.4	58.1	-	23.6
August 11-20	15.9	35.4	24.7	43.5	99.4	74.3	-	22.3
August 21-30	17.1	38.0	25.6	42.1	99.9	74.6	-	22.6
Sept 01-10.	16.2	36.9	25.3	36.9	93.7	65.4	-	21.5
Sept 11-20	14.2	32.2	21.6	53.6	99.9	79.1	-	20.4
Sept 21-30	8.7	27.5	15.6	47.2	99.9	85.7	-	16,5
Field climatic data for cauliflower cultures								
August 01-10	18.8	32.3	26.2	44.4	73.6	56.1	0	23.8
August 11-20	16.0	29.8	22.6	58.0	79.8	62.6	34.5	22.1
August 21-30	17.8	30.0	23.2	52.3	77.5	62.1	72.5	22.2
Sept 01-10	15.7	29.7	21.9	45.3	75.5	56.5	0	20.1
Sept 11-20	15.4	28.7	21.1	52.6	78.7	62.3	31.0	19.9
Sept 21-30	8.2	20.9	13.9	55.0	86.6	67.4	3.0	12.6

RESULTS

The treatments applied in the seedling stage had a favourable influence on the growth of tomato, cucumber and cauliflower plants.

In tomato seedlings the height of the plants, after 30 days from the emergence, was higher with 5.6%, the number of leaves with 20.0%, the number of roots / plant with 19.5% and the average length of the roots with 21.2% compared to the untreated control variant (Table 2, Fig. 1).



Fig. 1 Tomato seedlings; treated (left), untreated (right)

Table 2. Influence of Romagrobiofertil NP treatments at tomato, cucumber and cauliflower seedlings

Variant	Application date	Sowing date	Rising date	Plant high ²⁾ (cm)	Number of leaves ²⁾	Root number ^{2)/plant}	Root length ²⁾ (cm)
				%	%	%	%
Tomatoes							
V1 treated	02/20 03/18 ¹⁾	02/24.02	03/03	33.8	4.2	54.5	4.46
				105.6	120.0	119.5	121.2
V2 untreated	-	02/24	03/03	32.0	3.5	45.6	3.68
				100.0	100.0	100.0	100.0
Cucumbers							
V1 treated	03/18 04/12 ¹⁾	02/22	03/28	35.5	4.8	28.3	4.95
				117.9	123.1	140.8	133.1
V2 untreated	-	03/22	03/28	30.1	3.9	20.1	3.72
				100.0	100.0	100.0	100.0
Cauliflower							
V1 treated	02/18 03/18 ¹⁾	02/22	03/03	23.8	3.5	44.0	3.59
				107.7	129.6	124.6	128.2
V2 untreated	-	02/22	03/03	22.1	2.7	35.3	2.80
				100.0	100.0	100.0	100.0

¹⁾ 15 days after emergence; ²⁾ 30 days after emergence

In the case of cucumber seedlings the plant height was 17.9% higher, the number of leaves was 23.1% higher, the number of roots / plant was 40.8% higher and the average length of the roots was 33.1% longer (Table 2, fig. 2).



Fig. 2 Cucumber seedlings; treated (left), untreated (right)

A similar situation was found in cauliflower seedlings in which the plants height was higher with 7.7%, the number of leafs was 29.6% higher, the number of roots / plant was 24.6% higher and the average length of roots was 28.2% longer (Table 2, fig. 3).



Fig. 3 Cauliflower seedlings; treated (left), untreated (right)

The use of bio fertilizer after planting tomatoes and cucumbers in the greenhouse and cauliflower in the field has positively influenced the growth and development of plants, including the yields.

In tomatoes, the emergence of the first flower occurred 2 days earlier, the first fruit reached the normal size also 2 days earlier, 10% of the fruits ripened 2 days earlier while 80% of the fruits ripened 3 days earlier as compared to the untreated control lot (Table 3).

In cucumber the emergence of the first flower occurred 3 days earlier, as well as the first fruit when compared to the untreated control lot (Table 4).

Table 3. Influence of Romagrobiofertil NP on tomato blossoming and fructification

Variant	Rate (l/ha)	Number of applications	Planting date	Application date	First flower apparition	First fruit at normal size	10% fruits ripened	80% fruits ripened
V1 treated	15.0	3	08/09	08/09 08/20 09/19	08/16	09/02	09/12	10/15
V2 untreated	-	-	08/09	-	08/18	09/4	09/14	10/18

Table 4. Influence of Romagrobiofertil NP on cucumbers blossoming and fructification

Variant	Rate (l/ha)	Number of applications	Planting date	Application date	First flower appearance	First fruit appearance
V1 treated	15.0	3	08/09	08/09 08/19 09/17	08/14	08/18
V2 untreated	-	-	08/09	-	08/17	08/21

At the cauliflower crops, beginning of the inflorescence formation was with 3 days earlier and the inflorescence maturing with 3 days earlier compared with the untreated control variant (Table 5).

The favourable influence of the treatments with Romagrobiofertil NP on the growth of plants in the seedling phase and their growth and development after planting was also reflected in the obtained yield.

Table 5. Influence of Romagrobiofertil NP on cauliflower fructification

Variant	Rate (l/ha)	Number of applications	Planting date	Application date	Beginning of first inflorescence	Inflorescence maturing
V1 treated	15.0	3	08/09	08/09 08/24 09/23	09/22	10/15
V2 untreated	-	-	08/09	-	09/25	10/18

In tomatoes the yield was higher with 1.71 t / ha (3.16%) compared to the untreated control variant (Table 6). In cucumbers, the yield was higher with 1.47 t / ha (3.75%) and in cauliflower with 0.62 t / ha (2.43%) as compared to the untreated control variant.

Table 6. Influence of Romagrobiofertil NP fertilizer on tomato, cucumber and cauliflower yield

Variant	yield		Difference (t/ ha)	Signification
	t/ha	% compared with untreated variant		
Tomatoes				
V1 treated	55.47	103.16	+ 1.7	*
V2 untreated	53.77	100.00	-	-
LD 5 % =			1.386	
1 % =			2.546	
0.1 % =			5.628	
Cucumbers				
V1 treated	40.62	103.75	1.47	*
V2 untreated	39.15	100.00	-	-
LD 5 % =			1.030	
1 % =			1.892	
0.1 % =			4.182	
Cauliflower				
V1 treated	26.12	102.43	0.62	*
V2 untreated	25.50	100.00	-	-
LD 5 % =			0.454	
1 % =			0.835	
0.1 % =			1.846	

In all three species (tomatoes, cucumbers, cauliflower) the increase of yield was significant compared to the untreated control variant (Table 6).

CONCLUSION

1. The product Romagrobiofertil NP is an ecological fertilizer based on three species of bacteria: *Azospirillum lipoferum*, *Azotobacter chroococcum* and *Bacillus megaterium*.
2. The product has a favourable influence on the growth and development of plants both during the seedling stage and after planting.
3. In tomato, cucumber and cauliflower seedling the positive influence of the product has been achieved by increasing the height of the plants, the number of leaves / plant, the number of roots / plant and the length of the roots.
4. In tomato and cucumber crops in greenhouse and cauliflower in the field, the effect of the treatments was materialized both in the growth and development of the plants and in the yield increasing as compared to the untreated control variant.

REFERENCES

- [1] Sellamuthu Gothandapani, Soundarapandian Sekar and Jasdeep C Padaria (2017) *Azotobacter chroococcum*: Utilization and potential use for agricultural crop production: An overview. *Int. J. Adv. Res. Biol. Sci.* 4(3): 35-42
- [2] Steenhoudt, O. and Vanderleyden, J. (2000), *Azospirillum*, a free-living nitrogen-fixing bacterium closely associated with grasses: genetic, biochemical and ecological aspects. *FEMS Microbiology Reviews*, 24: 487–506
- [3] Attilla H. I., Alhasin A. M., Nasib M. A., Ghazali A. H., Zakaria L., Jais H. M., Balal I. A. A, Salleh B. (2010) Occurrence and microbiological characteristics of *Azospirillum* strain associated with leguminous and non- leguminous plants in Al Jabal Al Akhdar Eco region, Lybia. *American –Eurasian J. Agric. & Environmental Sciences* 8 (6): 617-625
- [4] Patel G., Singh S., Saxena S.K., Kaur K. J. (2016). Isolation, biochemical characterization and production of biofertilizer from *Bacillus megaterium*. *International Journal of Life Sciences Scientific Research* 2 (6): 749-752