WEED BIO-CONTROL AND TOMATO PLANTS GROWTH PROMOTION BY APPLYING AN ALTERNATIVE CULTIVATION SYSTEM INTO BIO-COMPOSITE MULCH

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ABSTRACT

Mulching represents a profitable technological step in crop production. Due to the cultural and efficacy advantages in solving certain agro-technical and environmental problems, mulching has expanded considerably in agricultural technologies. Using hairy vetch as cover crop mulch has the advantage to protect the soil during winter season against erosion and stores a larger amount of water in the active soil layer, while in vegetation season enriches the land in atmospheric nitrogen and reduces the weed development. The aim of this research was to develop an alternative mulch system for tomato cultures that synergically combines the benefits of a legume cover crop with the advantages of a biologically active film, which covers the mowed vetch. The bioactive layer contains useful micro-organisms that promote plant growth (like *Azospirillum brasilene* Sp001, *Bacillus amyloliquefaciens* B165) or that reduce the entomo-pathogen attack (*Beauveria bassiana*). This mulching system has proved to be useful in limiting the weed populations, in preservation and sustainable use of soil resources, in increasing fruit production and precocity, in improving photosynthetic efficiency and maintain a good plant physiology in unfavorable environment conditions, such as high temperatures and low relative humidity. The binding fruit percentage was with 3÷8% higher than the control, and fruit production was 6,8 kg/m^2 for variant inoculated with Sp 001, followed by the one with B165 with 6,4 kg/m^2, the reference being 5,2 kg/m^2. Plants grown in this bio-composite mulch system have a higher phothosyntethic efficiency, since the energy losses by fluorescence are lower. The mulched areas suppress weed populations whithin 36÷41 plants/m^2 compared to 94plants/m^2 in reference group. This study shows that the cultivation system in bioactive composite mulch for tomato culture, suppresses weed growing, improves culture physiology and increases fruit production.

INTRODUCTION

Modern agriculture embodies new knowledge and understanding of soil, biology and ecology of economic crops in the way of environmental conservation. Therefore, especially in the United States, the classic technologies are being replaced with different systems of planting crops in untilled or minimal tilled ground (Coughenour and Chamala, 2000). Mulches and cover crops, with their characteristics offer great advantages for the maintenance and improvement of the agricultural environment. Their benefits are reflected by the expansion of these agricultural practices both in sustainable cropping systems as in organic farming systems.

Among the benefits derived form mulching and cover crop technologies are the improvement of the chemical–physical and biological characteristics of the soil (Sarrantonio and Gallandt, 2003; Nakhone and Tabatabai, 2008), weed suppression (Hatcher and Melander, 2003), beneficial predators support (Pullaro et al., 2006, Clark 2007). Concerning the soil, the use of cover crops transformed into mulches decrease soil erosion and compaction, improves soil structure, improve rain water infiltration (Jones and Sing, 2000), water retention (Bhagat and Acharya, 1987; Anikwe et all., 2007), uphold soil moisture by reducing water evaporation from the field and help maintain stable soil temperature (Ji and Unger, 2001; Kar and Kumar, 2007), also enhance soil biological activity, efficiently accomplish the nutrient cycling with minimal external inputs (Steenhuis et all., 1998), significantly increase the amount of available phosphorus and potassium in the upper soil layer (Ortiz et all., 1992; Cadavid et al., 1998). If we refer to leguminous cover crops, these species have the ability to fix nitrogen from the atmosphere through symbiosis with rhizobia, increasing the fertility of the soil. It was demonstrated that using leguminous cover crop mulch the requirement for nitrogen fertilizers is reduced up to 50% (Sanju et. all., 2002) thus reducing the need for nitrogen fertilizers contribute to decrease nitrate leaking and greenhouse gas emissions from agriculture, like nitrous oxide emissions from soil, due to nitrogen fertilization (El-Hage Scialabba and Müller-Lindenlauf, 2010).

Organic mulch maintains a rich microflora and microfauna (Smith and Rose, 2001). This can be an advantage if we think at beneficial microorganisms, but, also, it can generate phytosanitary problems that could be easily avoided using chemical treatments, biological active compounds or antagonistic microorganisms.

Our studies propose an alternative system of mulch for growing tomatoes. By this bio-composite mulch technology we desired to overlap the benefits of organic mulching with the advantages of a bioactive film. As organic layer we selected the hairy vetch for its benefits as winter cover crop. The novel aspect in this technology is that we considered a second mulch layer, which we conceived with a special formulation that optimizes some of the vegetal mulch characteristics. The used microorganisms are the genera *Azospirillum*, *Bacillus* and *Beauveria*. The *Azospirillum* spp. is a good root colonizer that survives in harsh environmental conditions (Bashan et al. 2004). As plant growth promoter increases water and mineral uptakes from soil, within plants with properly colonized roots (Okon, 1984). *Azospirillum* is also cited to be effective as biocontrol agent, being able to reduce damping-off diseases caused by soil-borne fungal pathogens in tomatoes (Hadar et., 1986). In addition, *Azospirillum* can help plants minimize the negative effects of abiotic stresses (Barassi et al., 2007). The *Bacillus* strains are mainly known for their ability to biologically control various plant diseases in agricultural plants, and have been used in several biological pesticide formulations. *Bacillus* spp. produce at least 66 different antibiotics (Katz and Demain, 1977). *Bacillus* spp. is a plant growth-promoting rhizobacteria, used to stimulate plant growth by phytohormone production (Bastian, 1998; Gutierrez-Manero, 2001) and capability of phytate solubilisation (Bajpai et al., 1971; Gupta et al., 1994; Idris, 2004). *Beauveria bassiana*, as an entomopathogenic fungus, has been selected for its potential use in suppressing insect populations.

Our targets were: to develop a technology for obtaining bio-composite mulch (vegetal mulch improved with a covering bioactive film), obtaining a technology for growing tomatoes in this alternative system of mulching, and performing studies on the multiple effects of alternative agriculture in the bio-composite mulch regarding tomato production and quality of crops.

MATERIALS AND METHODS

Placement and experimental design

The research was conducted in the experimental field of Research and Development Institute for Plant Protection - Bucharest, on an area of 250 m^2. Previously the system was tested and optimized in controlled environmental conditions (growing chamber). This topic was aimed in our ASTARTE project which was a research assignment sustained by the Education and Research Ministry.

To achieve the proposed alternative system we reared as wither cover crop the hairy vetch *Vicia villosa* which we converted in a mulch layer. The selected leguminous cover crop served as a good precursor, protected soil texture, improved soil structure, and enriched soil fertility by converting in available forms the phosphorus and potassium means and atmospheric nitrogen into nitrogen fertilizers with their nodule formation in symbiosis with *Rhizobium* bacteria, the wild or selected forms. Mowed hairy vetch was covered by a second layer consisting of biologically active films. Fundamentally this bioactive film contains:

- selected microorganisms which confer biological activity to the mulch film. These microorganisms, such as plant growth promoting rhizobacteria (PGPR) from *Azospirillum* and *Bacillus* genera, and entomological-pathogenic-micromycet *Beauveria bassiana;*
- co-polymer hydrogels, which improve water retention capacity, and afterwards slowly release it to plant needs also serving as support for sustaining microorganism viability;
- granular organic mulch, which helps maintain stable soil temperature, soil moisture and improves weed control;
- high performance tackifier with reticular binds that confer high flexibility to the film composition and tie together the mulch components, allowing high water penetration through the layer and, when employed, it reduces clogs in the machine. Because of the wetting, the mulch slurry becomes very slippery, helping mulch to flow more easily through the machine and also it lubricates the applying system allowing uniform distribution, similar to a film;
- specific adhesives that sustain the compactness and elasticity of the layer.

The land was tillage in previous fall, plowed and three way disk harrowed. The cover crop hairy vetch was manual seeded in the first decade of October 2007, in 15 cm spaced rows. Experimental plot size was 30m / 8,3m were the experimental variants had 4.2 m long and 2.1 m wide. The alleys were 1m, and 0,8m wide. Experimental scheme anticipated the randomness of the tomato culture, and so the cover crop was seeded only in the mulch predicted plots. Experimental scheme had randomized blocks and included six variants in three repetitions, after the Latin rectangular randomization scheme (see Randomization Scheme).

Randomization Scheme

R1	V4 V5 V6 V1 V2 V3	B1
R2	V2 V3 V4 V5 V6 V1	B2
R3	V1 V2 V3 V4 V5 V6	B3

Legend: B – block R – repetition V - variant

In the next spring the hairy vetch was transformed into mulch using a two knives mower C2C (INMA design) carried by a 45HP tractor, and driven by the tractor's PTO at 540rpm speed. The mowing was done on the 15th of May 2008, at the beginning of hairy vetch blossoming. The culture had 50-70cm height and was cut to les than 5cm above the soil. The mulch layer was in this way obtained *in situ*. After two days of drying, the vegetal layer was covered with bioactive film.

The bio-composite mulch was applied in 5 compositional variants, comparing to a reference without mulch, as follows:

V1 - composite mulch without added microorganisms (CM);
V2 - bio-composite mulch + *Azospirillum brasilense* Sp 001 (CM.Az);
V3 - bio-composite mulch + *Bacillus amyloliquefaciens* B165 (CM.B);
V4 - bio-composite mulch + *Azospirillum brasilense* + *Bacillus amyloliquefaciens* (CM.A+B);
V5 - bio-composite mulch + *Azospirillum brasilense* + *Bacillus amyloliquefaciens* + *Beauveria bassiana* BbLd1/04 (CM.A+B+Bv);
V6 – reference group (without mulch, WM).

Preparation of bioactive layer that covers the hairy vetch mulch

The bioactive film represents the novelty of the mulching system had the following components:

· Turbosorb - that is a co-polymer gel, which improves water retention with 50% and slowly releases it over time, making rain and irrigation water by available to plants for longer time period. This hydrogel also improves microorganism maintenance.

· TurboTack - is a high performance tackifier with reticular binds that tie together mulch components, allow the slurry to uniform distribution, and confer high flexibility to the film. Water can easily penetrate this layer. And the component is environmental safe and totally biodegradable in 3 to 6 months.

· Jet Spray fiber mulch – emulate poly-fibers but is based on cellulose fibers, which are biodegradable. This component was designed to improve the performance of jet agitated hydraulic mulching machines. It is homogenous, weed free, and within the mulch layer helps regulate soil temperature, and suppresses the weeds.

· Disodium tetraborate and soluble starch – which serve as biodegradable adhesives.

For preparation, in 1/8 of the water quantity, preheated at 40°C, some of the constituents were added in rain, in a continuous slow stirring, as follows: Turbo Tack, soluble starch, disodium tetraborate, Turbosorb, and microorganism cultures, depending of the mulch version. This composition was continuously slowly stirred for about 1 hour. Meantime the fiber mulch was wetted in the remained water, and finally the two compositions were mixed together.

Microbial preparation for activating the biocomposite mulch

The biological activity of the mulch is given by the beneficial microorganisms: *Azospirillum brasilense* and *Bacillus amiloliquefaciens*, plant growth promoters and *Beauveria bassiana* entomopathogenic fungi.

Their cultivation was performed in a continuous flux technology, cascade type, which required several steps:

- freshen up the pure culture inoculum;
- preparing laboratory inoculum;
- multiplying in Erlenmeyer flasks;
- with the possibility of further growing in bioreactors, if needing large a*months* of microbial inoculum for biological activation of mulch required for hectares;
- biomass separation from culture medium.

Process technology of obtaining *Azospirillum brasilense* Sp 001

· Bacterial strain from the RDIPP microbial collection was freshened up on Döbereiner medium (table 1) and incubated one week at 30°C.

· Microscopically examined pure culture was used for laboratory inoculum preparation. Biomass multiplication was made in small Erlenmeyer flasks with 50ml volume of tailings in which were added 15 ml of AFM_{20} medium (table 1) and 1ml of bacterial inoculum. Flasks were then incubated in a horizontal shaker at 150 rpm at 30°C environment. After 24 hours, samples were aseptically and microscopically examined to verify the purity of culture.

· Biomass multiplication in large Erlenmeyer flasks was made to produce the inoculums amount necessary for the bioactive mulch. For this purpose Erlenmeyer flasks of 500ml volume of tailings were filled with 150 ml of AFM_{20} medium inoculated with 15ml of previous bacterial inoculum. Flasks were incubated at 30°C with 150 rpm shaking, for 2 days.

· The last step of producing the bacterial biomass is the separation of bacteria from the culture medium. The nephelometry test showed that the *Azospirillum* culture yielded 11,3g/L biomass. The culture was spun at 10.000 rpm and the resulted pellet was dispersed in phosphate saline buffer (PBS: 8g NaCl; 0,2g KCl; 1,44g Na_2HPO_4; 0,24g KH_2PO_4; up to 1000ml distilled water; pH 7,4; autoclaved 15 minutes at 121°C) until it reached 10^6 cfu/ml concentration.

Process technology of obtaining *Bacillus amiloliquefaciens* B 165

· One vial of the lyophilized bacteria was carried out from the RDIPP microbial collection storing freezer and brought to room temperature. The ampoule was aseptically opened and the bacterial biomass was suspended in 1 ml of sterile PBS in an eppendorf tube. The eppi was vortexed for better homogeneity. Fifty micro litters were poured in Petri dishes on Luria Bertani – agar medium (bactotryptone 10 g/L, yeast extract 5 g/L, sodium chloride 10 g/L, 1L distilled water, pH 7.5, autoclaving 15 minutes at 121°C), and displayed using exhaustion loop technique in order to obtain pure culture isolated colonies. The plates were incubated over night at 28°C.

· The laboratory inoculum was prepared in test-tubes inoculated with previously obtained pure culture colonies. The test-tubes with the B 165 strain were incubated at 28°C for 48 hours then were examined macroscopically to assess cultural purity (typical morphology of *B. amiloliquefaciens* presents rough-looking colonies).

· The biomass multiplication in Erlenmeyer flasks was done in order to produce the bacterial inoculum amount for the bioactive mulch. The flasks used had 500ml volume of tailing, and were filled with 150 ml of LB broth medium inoculated with previous obtained bacterial inoculum. The incubation was made at 28°C in 150 rpm shaking, for 2 days.

· *Bacillus* biomass was separated by centrifugation at 6.000 rpm and dissolved in PBS, to a concentranion of 10^8 cfu/ml.

Beauveria bassiana BbLd1/04 inoculum was prepared by the mycology laboratory of RDIPP.

Table 1

Composition of the growth culture media used for in obtaining microorganism biomass

AFM $_{20}$		**Döbereiner**	
$CaCl_2$ x $2H_2O$	20 mg	$CaCl_2$ x $2H_2O$	20 mg
NaCl	100 mg	NaCl	100 mg
$MgSO_4$ x 7 H_2O	200 mg	$MgSO_4$ x 7 H_2O	200 mg
$FeSO_4$ x 7 H_2O	10 mg	Na_2Mo_4 x 2 H_2O	20 mg
Na_2Mo_4 x 2 H2O	2 mg	K_2HPO_4	600 mg
K_2HPO_4	600 mg	KH_2PO_4	400 mg
KH_2PO_4	400 mg	Malic acid	5 g
Yeast extract	3 g	KOH	4 g
Glucose	15 g	$FeCl_3$ x $3H_2O$	16 mg
		Bromthimol blue – aqueous solution 0,5%	2 ml
Distilled water	1000 ml	Agar	2 g
		Distilled water	1000ml

Tomato culture and biometric observations

The tomato culture was established one day after the bioactive film was sprayed above the hairy vetch mulch. Planting scheme used equidistance lines, with distance between rows of 70 cm and distance between plants in row of 35 cm, using Dacia variety of *Lycopersicum esculentum* seedlings.

During growth the culture was groomed using specific technology. A series of observations and measurements were made to highlight the influence of bio-composite mulch on tomatoes growth and fructification. The observations aimed at studying: growth dynamics in plant height, distance pending the first inflorescence, inflorescence's sequence, the average number of flowers in inflorescence, the average number of fruit in floret, the percentage of bended fruit, fruit production and biochemical fruit composition.

Weed evaluation

For weed evaluation we monitored the number and species of the weed spectrum in each variant and repetition, avoiding unrepresentative areas. In our method we used a metric frame of 0,5m on side (which means 0,25m^2). Metric frame was randomly applied two times in all repetition of each variant. Weed infestation was reported to m^2 of variant. Weed efficacy was calculated using Abbott formula (1925):

$$Corrected\% = \left(1 - \frac{weed\_number\_in\_mulched\_\mathrm{var}iant}{weed\_number\_in\_Control}\right) \times 100$$

Environmental condition

Environmental conditions were monitored with a meteorological station placed in the experimental plot proximity. The weather station could monitor 9 parameters, of which more important are the air temperature, relative humidity, leaf wetness, precipitations and solar radiation.

Monitoring pest agents

Climatic conditions are essential in technological crop managing, including pest control. In present there are several programs that can forecast pathogen attack within cropped plants. TOMCAST (TOMato disease foreCASTing) a computer model based on field data attempts to predict fungal disease development, namely early blight, septoria leaf spot and anthracnose on tomatoes. This program algorithm is based on Disease Severity Values (DSV) that represents measure units given to a specific increment of disease (early blight) development. In other words, a DSV is a numerical representation of how fast or slow disease (early blight) is accumulating in a tomato field. The DSV is determined by two factors; leaf wetness and temperature during the "leaf wet" hours. As the number of leaf wet hours and temperature increases, DSV accumulate at a faster rate (Table 2).

Using TOMCAST algorithm when tracking the environmental parameters we could prevent phytophatogen attack and monitor pest pressure infection of the *Phytophtora infestans*, *Septoria lycopersici*, *Fulvia fulvum* cf. *Cladosporium fulvum* and *Alternaria solani*.

The DSV index is in positive correlation with the increasing temperature and high relative humidity ("leaf wet" hours), since their levels increase, DSV value is also higher. Conversely, when there are fewer leaf wet hours and the temperature is lower, DSV accumulate points slowly if at all. When the total number of accumulated DSV exceeds a preset limit, called the spray interval or threshold, a fungicide treatment is recommended to protect the foliage and fruit from disease development. The spray interval can range between 15-20 DSV.

To prevent pathogen attack we daily monitored the ambient conditions. We decided to apply fungicide treatments when accumulating 18-20 DSV points. After 18 DSV points were reached we applied the first phytosanitary treatment. We retook DSV count at the end of the treatment break, when the treatment effectiveness was ended.

Table 2

Condition that contributes to the increase of DSV values

Average temperature during leaf wet hours	**Hours of leaf wetness per day**				
13 – 17 ºC	0 – 6	7 – 15	16 – 20	21+	
18 – 20 ºC	0 – 3	4 – 8	9 – 15	16 – 22	23+
21 – 25 ºC	0 – 2	3 – 5	6 – 12	13 – 20	21+
26 – 29 ºC	0 – 3	4 – 8	9 – 15	16 – 22	23+
Daily DSV =	**0**	**1**	**2**	**3**	**4**

Table 3

Summary of morphometric measurements at 3 weeks after planting (9th June 2008)

Variant		Plant size (cm)	Plant stalk diameter (mm)	Inflorescences no. / plant	Flowers and buds no. / plant
V1	**CM**	26,92	6,97	**1,77**	8,25
V2	**CM.A**	**28,92**	6,99	**2,58**	**12,33**
V3	**CM.B**	28,75	6,67	2,33	11,86
V4	**CM.A+B**	28,42	**7,04**	2	9,65
V5	**CM.A+B+Bv**	27,42	6,67	2,33	9,75
V6	**WM**	**26,83**	**6,63**	1,92	**8**

Fluorescence measurements

The measurements of chlorophyll fluorescence (CF) were shown to be valuable tools in plant physiology. CF can be used as an early indication of many types of plant stress (Anderews et al., 1995; Janssen and van Hasselt, 1994; Mohammed et al., 1995) including temperature stress (Larcher, 1994; Yamada et al., 1996).

CF is a nonradiative energy dissipation emitted by autotrophic plants, and originates mainly from photosystem II (PS II). An increase in this energy rate is one of the first responses of a plant to environmental stress (Screiber et al.,1994). High a*months* of reflected CF competes photosynthesis in PS II, and generates non-photochemical quenching by enhancing the radiation-less deexcitation of the energized thylacoid membrane (Krause and Weiss, 1984; Bilger and Schreiber, 1986). This means that CF emission is mainly correlated with two parameters: chlorophyll content and photosynthetic activity of leaves. If the F690/F735 ratio is higher than the chlorophyll content is lower and / or the process of photosynthesis is decreasing (e.g. high environmental temperature, damaged leaf, adverse effects caused by herbicide or other stress factors). Radiation quality (in red or blue) does not affect CF index.

Chlorophyll fluorescence index is shown by the ratio between leaf emitted fluorescence measured at two specific wavelengths: 690nm and 735nm respectively. Because F690/F735 fluorescence ratio is a good indicator of stress in the plant it can be used to detect plant physiological states in crops that are monitored by airborne systems (because those systems can cause laser-induction of CF and read it simultaneously).

Our measurements of chlorophyll fluorescence were made using an optic fiber fluorimeter, USB 2000, from Oceans Optics. We analyzed 4 mature leaves of 4 plants from each variant repetition, at tree weeks after the seeding.

Porometry measurements

Porometry is studying gas exchange between plant leaves and the environment trough stomata aperture. Stomata ostiole is the dominant factor in the diffusion conductance of leaf surfaces, which controls both water loss from plant leaves and uptake gases for photosynthesis and respiration. Measurements of the diffusion conductance are therefore important indicators of plant water status and provide a valuable insight into plant adaptation to environmental variables. Stomatal pores indulge a balance that unleashes the pressure difference, produced in the diffusion paths, between the dry air from the atmosphere and tissue humidity.

If the CF determinacy is a reliable method for monitoring heat stress because it is not influenced by stomata closure induced by heat, we can say that the porometric method is efficient in monitoring hydric stress. Correlating this information with the physiological status of plants and we can determine the effects of the mulching methods on cropped plants.

In our study we measured stomata conductance and transpiration rates of 3-4 tomato leaves at 8 weeks after seeding, using AP4-UM-3 Dalta T device porometer from Cambridge, UK.

Before measurement the device was calibrated using a calibration plate, prepared in advance with at least one hour prior to the readings, in order to stabilize its humidity. This plate has remained relatively stable in terms of humidity during measurements are taken with a gap of ± 2% (falling to within

± 5%). Recalibration of the relative air humidity was rebuilt every 10-15 minutes during the performed measurements. Measurements were compared with a calibration plate of known resistance in order to derive the stomata resistance or conductance of the leaf.

RESULTS

This mulching technology led to positive culture reactions for the tomato plants both in terms of vegetative growth and fructification.

Growth dynamics of three weeks old tomato plants reviled that the bio-composite mulch enriched with *Azospirillum brasilense* Sp001 increased by 7,2% plant size comparing to the non-mulched reference, and about 7% comparing to non-bioactive mulch. The two references were also inferior, in terms of generative development, to bio-composite mulched variants that presented a moderate precocity. In this regard, in the second and third variants, those with *Azospirillum brasilense* Sp001 and *Bacillus amyloliquefaciens* B165, the number of flower and buds was higher especially by the cause of the advanced generative formations.

After 2 months of cultivation it can be seen that plant size is higher in all bioactive and composite mulches comparing to the un-mulched reference. Other vegetative parameters were not significantly influenced. The improvement of biometric characteristic (table 4) is estimate do to the decomposition of the hairy mulch and plant growth promotion conferred by the used bacterial strains.

Table 4

Biometric indicators of tomato plants after 2 months of cultivation (21st July 2008)

Variant		Average no. of leaves pending the first inflorescence	Plant lenght pending th first inflorescence (cm)	inflorescence's sequence	Averaged plant high (cm)
V1	**CM**	5,1	20,06	2,7	77,2
V2	**CM.A**	4,5	18,44	2,6	72,7
V3	**CM.B**	4,7	19,57	2,3	75,2
V4	**CM.A+B**	4,3	19,95	2,7	70,6
V5	**CM.A+B+Bv**	4,6	21,69	2,6	69,8
V6	**WM**	5,2	22,4	2,8	63,4

Referring to fructification process (table 5), we can say that bio-active composite mulch variants positively influenced the flower formation, between 14% in CM.A, 13% in CM.A+B, and 12% in CM.B above the WM reference. The V1 of CM was not meaningfully high compared to WM control. As consequence, due to the excessive high temperature during the vegetation, flower formation significantly decreased in the upper inflorescences.

Table 5

Flower formations during the vegetation season

Variant		Infl. I	Infl. II	Infl. III	Infl. IV	Infl. V	Infl. VI	Infl. VII
V1	**CM**	4,5	4,8	5	4,4	4,1	3,4	3
V2	**CM.A**	4,6	5	5,2	5,1	4,8	3,6	3,4
V3	**CM.B**	5	5,2	5,3	5	4,3	3,2	3,3
V4	**CM.A+B**	4,7	5	5,1	4,9	4,4	3,4	3,8
V5	**CM.A+B+Bv**	4,2	4,3	4,5	4,8	4,5	3,5	3
V6	**WM**	4,1	4,2	4,8	4,7	4	3,4	2,6

* Abbreviation: Inflorescence - infl.

Fruit binding process was stimulated in bio-active composite mulches, due to the mulch characteristics. However, thermal unfavorable environment, commonly above 30°C, affect pollen germination, and so, decreased fruit binding process (table 6).

Table 6

Fruit binding process during the vegetation season

Variant		Infl. I	Infl. II	Infl. III	Infl. IV	Infl. V	Infl. VI	Infl. VII
V1	**CM**	3,1	3,5	4	2,5	1,5	1,2	1,3
V2	**CM.A**	3,5	4	4,2	2,6	1,6	1,1	1,3
V3	**CM.B**	3,2	3,6	3,7	2,5	2,1	1,2	1,8
V4	**CM.A+B**	2,8	3,2	4	2,7	1,3	1,1	1,6
V5	**CM.A+B+Bv**	2,6	2,8	3,4	2,7	1,6	1,1	1,3
V6	**WM**	2,4	2,3	2,9	2,6	1,5	1	1,1

Fruit binding percentage was improved by the bio-active mulch composition but the unfavorable environmental conditions negatively influenced this process (table 7).

Table 7

Fruit binding percentage

Variant		Infl. I (%)	Infl. II (%)	Infl. III (%)	Infl. IV (%)	Infl. V (%)	Infl. VI (%)	Infl. VII (%)
V1	**CM**	67,86	72,41	80,00	57,09	35,83	33,83	44,62
V2	**CM.A**	76,00	79,31	81,48	51,85	33,04	31,04	39,57
V3	**CM.B**	64,00	69,23	69,23	49,00	48,42	37,42	53,75
V4	**CM.A+B**	59,26	64,29	77,78	54,17	30,00	30,00	40,95
V5	**CM.A+B+Bv**	61,11	65,52	75,00	56,67	36,00	31,00	43,33
V6	**WM**	58,33	54,35	60,87	55,00	38,14	30,14	39,67

The smallest percentage of fruit binding was obtained in the last two formed inflorescences, when the high temperatures associated with air dryness have contributed to flowers abortion. However, an increase in the percentage of fruit binding to the seventh inflorescence has been noticed, but the number of flowers in cyme was very small and the formed fruits were of lower quality.

The fruit production achieved best results in V2 followed by V3, where productivity reached 1,7 kg /plant in CM.A and 1,6 kg /plant in CM.B. The third variant, consisting of CM.A+B, given 1,5 kg /plant. The other mulched variants caused a production of 1,4 kg /plant. The reference recorded the lowest production, 1,3 kg / plant (table 8).

Table 8

Tomato productivity on different types of mulching systems compared with bare ground

Variant		Tomato productivity	
		Kg/plant	**Kg/m^2**
V1	**CM**	1,5	6
V2	**CM.A**	1,7	6,8
V3	**CM.B**	1,6	6,4
V4	**CM.A+B**	1,5	6
V5	**CM.A+B+Bv**	1,4	5,6
V6	**WM**	1,3	5,2

Weed evaluation

Tomato crop is vulnerable to weed competition. The frequencies of unwanted plants in cropped tomatoes is due to the weed aggressiveness for water and nutrient competition, unfavorable environment that they can create by decreasing light conditions and wield pathogen infections, sustain infections sources. After mapping sagittal weed flora, presented in table 9, we remarked 4 predominant weed species: *Convolvulus*, *Cirsium*, *Sorghum* and *Xanthium*. This weeds have biological particularities that helps their survival, like vegetative buds present on their roots, rhizomes, grate penetrative strength in germination or rapid growth and spreading-ness that it can 'choke out' the cash crops that have been planted.

Table 9

Mapped sagittal weed flora

Variant	**Sagittal flora**					**Observations**
	Dicotyledoneae			***Monocotyledoneae***		
	Genul/Specia	**Weed/m²**		**Genul/Specia**	**Weed/m²**	
V1 CM	*Atriplex hastate*	1	29	*Digitaria*	9	*Sorgum halepense* has been numerical representative between monocotyledon weeds.
	Convolvulus spp.	11		*Sorgum halepense*		
	Cyrsium arvense	1				
	Geranium ssp.	3				
	Polygonum aviculare	2				
	Veronica persica	6				
	Xanthium strumarium	5				
V2 CM.A	*Atriplex hastata*	1	31	*Digitaria*	8	–
	Convolvulus arvensis	23		*Setaria*		
	Cyrsium arvense	1				
	Veronica persica	4				
	Xanthium strumarium	2				
V3 CM.B	*Anchusa officinalis*	4	28	*Sorgum halepense*	9	*Sorgum halepense* has been numerical representative between monocotyledon weeds.
	Convolvulus ssp.	21		*Setaria*		
	Cyrsium arvense	1		*Digitaria*		
	Polygonum aviculare	1		*Echinochloa crus-galli*		
	Xanthium strumarium	1				
V4 CM.A+B	*Amaranthus spp.*	1	27	*Setaria*	9	–
	Capsela bursa-pastoris	1		*Digitaria*		
	Convolvulus ssp.	13				
	Cyrsium arvense	6				
	Veronica spp.	3				
	Xanthium strumarium	3				
V5 CM.A+B+Bv	*Atriplex hastata*	1	30	*Setaria*	11	–
	Brassica juncea	1		*Digitaria*		
	Convolvulus ssp.	12				
	Cyrsium arvense	3				
	Geranium ssp.	1				
	Matricaria spp.	2				
	Polygonum aviculare	1				
	Veronica persica	3				
	Xanthium strumarium	6				
V6 WM	*Brassica juncea*	2	55	*Digitaria*	39	- *Sorgum halepense* has been numerically representative for monocotyledon weeds. - Common wheat, developed from samurasler, has been noted as weed in this culture.
	Convolvulus arvensis	29		*Setaria*		
	Cyrsium arvense	11		*Sorgum halepense*		
	Geranium ssp.	3				
	Xanthium strumarium	10		*Triticum aestivum*		

* - The sixth variant represented the reference without mulch, and has been subjected to tillage technology.

By mulching the cropped soil weed influx was significantly lower compared with the control (table 10).

Table 10

Mulch efficacy comparing with the reference

Variant		Weed no./m^2	Weed percentage comparing with non mulched control	Efficacy towards the not mulched reference (%)
V1	CM	38	40,4 %	59, 6
V2	CM.A	39	41,5 %	58,5
V3	CM.B	37	39,4 %	60,6
V4	CM.A+B	36	38,3 %	61,7
V5	CM.A+B+Bv	41	43,6 %	56,4
V6	WM	94	100 %	0

Bioactive ingredients of mulches significantly influenced weed management comparing with the reference, reducing weed number. Weed percentage in mulched variants ranged from 38,3% to 43,6% compared with control. Mulch effectiveness has been considered good for a sustainable agricultural system without herbicides.

Chlorophyll fluorescence determinations on tomato leaves

Chlorophyll fluorescence CF emission is mainly correlated with chlorophyll content and photosynthetic activity of leaves. Higher F690/F735 ratio is measured when chlorophyll content is lower and / or photosynthesis process decreases.

CF measurements presented in table 11 show higher values of the fluorescent emission in the non-mulched reference (V6) and not bio-activated mulch (V1). The high a*months* of reflected CF indicate energy loss. Those energy loses reflect plant response to environmental stress.

The microorganisms included in the bioactive film protect plants against bio-climatic stress. The energy lost by fluorescent emission is reduced to half in *Azospirillum* inoculated variant versus control, and significantly decreased for plants cultivated in CM.A+B. Photosynthesis is improved also on the other bio-activated mulches.

With this CF measurement was revealed that, used microorganisms not only improve plant nutrition but also protects them against bio-climatic stress factors.

Porometric measurements

The stomata conductance values and rate of leaf transpiration recorded by the porometer were centralized in table 12. The measured values indicate that the above mentioned two parameters were higher in the bio-active mulch compared to reference and biologic inactive mulch.

Since stomata provide gas exchange between plant tissue and environment, the time when stomata ostioles are opened is extremely important in the physiological processes of plants. Carbon dioxide from the atmosphere, necessary in photosynthesis and the oxygen needed for respiration processes, enter the tissue mostly trough this stomata apertures. A better conductance of those molecules in the system increases plant photosynthetic processes. Also, water exchange through stomata is permitted so plants could adjust their temperature and better adapt to environmental conditions.

Porometric analyses revealed that gas and water exchange is improved in bioactive mulched variants as they contain microorganisms, and plants could better adapt to environmental conditions even on high temperature and low water precipitation as were registered in the vegetation period.

The presence of *Azospirillum brasilense* Sp001 and/or *Bacillus amyloliquefaciens* B165 bacteria in bioactive composite mulches caused significant increase of stomata conductance. This means that those mulching variants increased the agronomic resources. Because stomata conductance is positively correlated with photosynthetic capacity also means that the bioactive variants of mulching increase the photosynthetic process efficiency. Stomata conductance measured in plants from bioactive mulches reached values of 0,25 – 0,26 moli•m^{-2}•s^{-1}. For the reference without mulch stomata conductance was 0,21 moli•m^{-2}•s^{-1}. Leaf transpiration rate was also low in control, about 4,07 mmoli H_2O•m^{-2}•s^{-1}.

In bioactive variants stomata aperture allowed a transfer of water vapors between 4,41 up to 4,53 mmoli H_2O•m^{-2}•s^{-1}. Biocomposite mulch application thus increases agronomic means, providing higher amount of water in the active layer due to the soil coverage and improving photosynthesis activity.

Table 11

Chlorophyll fluorescence rates of tomatoes after 3 months from planting (18th of august 2008)

Variant	F 735 nm	F 690nm	F 690 / F 735
V1R1	0,7474	0,30498	0,408055
V1R2	1,6726	0,17752	0,106134
V1R3	1,2329	0,19378	0,157174
V1-CM			**0,2238**
V2R1	2,0772	0,28061	0,135091
V2R2	1,9936	0,16577	0,083151
V2R3	1,3014	0,14496	0,111388
V2-CM.A			**0,1099**
V3R1	1,1333	0,38751	0,341931
V3R2	1,5285	0,12042	0,078783
V3R3	1,2154	0,15297	0,12586
V3-CM.B			**0,1822**
V4R1	1,916	0,30899	0,161268
V4R2	0,74047	0,14302	0,193148
V4R3	1,7183	0,1314	0,076471
V4- CM.A+B			**0,1398**
V5R1	1,6588	0,2869	0,17295
V5R2	1,6533	0,3273	0,197924
V5R3	1,7963	0,3381	0,1882
V5- CM.A+B+Bv			**0,1864**
V6R1	0,71756	0,16101	0,224367
V6R2	0,82858	0,16891	0,203855
V6R3	1,6021	0,1585	0,263103
V6-WM			**0,2305**

Table 12

Porometric measurements registered between 12-14pm on 18th of July 2008

Variant		Stomatal conductance <moli•m^{-2}•s^{-1}>	Leaf transpiration rate <mmoli H_2O•m^{-2}•s^{-1}>
V1	**CM**	0,23	4,09
V2	**CM.A**	0,26	4,53
V3	**CM.B**	0,25	4,41
V4	**CM.A+B**	0,25	
V5	**CM A+B+Bv**	0,25	4,51
V6	**WM**	0,21	4,07
DL5%		0,02	0,34

CONCLUSIONS AND RECOMMENDATIONS

Bioactive composite mulch system that we have developed exhibits great benefits on agronomic values and tomato cropping. The findings of our research were:

- using hairy vetch, a winter cover crop technology, we ensured a large quantity of vegetative biomass production that can provide a grate organic layer, and also, within decomposition, it gradually delivers nutrients to the subsequent crop;
- the hairy vetch improves soil fertility, increasing nitrogen amounts in the rhizosphere;
- the bioactive ingredients, represented by plant growth promoting bacteria, convert nutrients in soluble forms so they can be easily absorbed in plants;
- the winter crop protects soil against erosion and improves its texture and hydro-physical characteristics;
- the organic layer is easily converted into mulch using conventional mowers or different types of roller crimpers;
- the mulching technology is improved by the bioactive composition bringing various benefits onto the cash crop such as increasing water and mineral uptakes from soil, providing phytostimulation and phytosanitary protection;
- the composition of bioactive layer, which covers the organic mulch, includes not only useful microorganisms but also binding agents which confer flexibility for better coating and durability over the vegetative period; the bioactive layer also contains fillers for density, protection agents against erosion and hydrogels that store large quantities of water releasing it when needed by plants;
- the bioactive mulching layer can be uniformly sprayed over, in a thin layer;
- the bioactive biocomposite mulch system reduces weeds within the cash crop, and confers to the tomato culture a better environmental protection against stress agents;
- the technology we used leads to positive yields on tomato, both in terms of vegetative growth and fructification as well as conferring precocity.

Technological results demonstrate that the bioactive composite mulch can combine the benefits of hairy vetch mulch with the advantages of a bioactive film in a synergic way, conferring to the cash crop some important agro-technological advantages such as managing weeds and providing water for longer time periods as well as conferring yield and precocity in tomato cultures.

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