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HYDRO-GELIFIED AND FILM FORMING FORMULATION OF MICROBIAL PLANT BIOSTIMULANTS FOR CROP RESIDUES TREATMENT ON CONSERVATION AGRICULTURE SYSTEMS

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ABSTRACT: Microbial plant biostimulants represent one of the solutions for the negative impacts associated with high residues agricultural systems. We developed a hydro-gelified and film forming formulation, which we used for *in situ* formation of a bio-composite mulch, by treatment on crop residues. Into this hydro-gelified and film forming composition we included microbial biostimulants strains, *Trichoderma asperellum* T36 and *Brevibacillus parabrevis* B50. We tested the effects of application of such microbial plant biostimulant formulation on polyamines and ortosilicic acid release from a mixture of hairy vetch and corn residues, on controlled conditions. We evaluated the influence of crop residues treatment with our formulation on crop yield and soil characteristics - water stable aggregates, glomalin related soil proteins. The results demonstrate that treatments on crop residues with our formulation of microbial plant biostimulants compensates some disadvantages of high residues conservation agriculture, enhancing its effects on soil aggregate stability, due to the promotion of glomalin related soil proteins formation.

Keywords: microbial formulation, high residues farming, polyamines, silicon, glomalin related soil proteins

INTRODUCTION:

Conservation agriculture systems involve less tillage intervention and soil coverage with crop residues (Kassam et al., 2009). Such systems present advantages related to soil erosion and nutrient leakage (Hobbs et al., 2008), which make them very suitable for wetland ecosystems (Palm et al., 2014). Permanent soil coverage is an important principle of conservation agricultural (CA) systems (Chivenge et al., 2007). During vegetation of cash crop the coverage is assured by plant residues layered on soil surface, resulted from a previous crop, which is left anchored or loose after harvest, from a cover crop grown and killed to provide mulch, or from externally applied mulch (Govaerts et al., 2009; Hobbs et al., 2008). Plant residues covering the soil limit water evaporation, promote water infiltration, enhance soil structure formation, facilitate accumulation of soil organic matter, reduce erosion and decrease soil temperatures (Fabrizzi et al., 2005; Franzluebbers, 2010; Madari et al., 2005). The importance of plant residues coverage lead to the formulation of two new principles added to the initial three principles of CA systems (Scopel et al., 2013): (*i*) producing biomass whenever possible (and recycling water / nutrients, which otherwise will be lost, by using plant residues as a nutrient reservoir) and (*ii*) introducing multifunctional cover crop, which allow mulching the soil permanently.

Despite many advantages, there are also negative impacts associated with high residue systems. Plant residues promote the development of soil-borne pathogens (Bockus and Shroyer, 1998), including devastating one like those producing *Fusarium* head blight (Guo et al., 2010; Leplat et al., 2013). High level of plant residues could reduce nitrogen availability for cash crop on early development stages (Geisseler et al., 2010). In temperate regions plant residues could have a detrimental effect on crop establishment and early growth of crop, mainly due to soil temperature reduction (Kravchenko and Thelen, 2007). Reduced soil tillage, which is necessary for keeping high plant residues coverage on soil, generate disadvantages related to less aerated soil structure (Page et al., 2013).

Plant biostimulants represent an emerging class of agricultural inputs (du Jardin, 2015). Microbial plant biostimulants, an important category of this emerging class of agricultural inputs, promote plant growth, enhances / benefits nutrients uptake and activate plant systemic response against biotic and abiotic stress (Calvo et al., 2014). Among microbial plant biostimulant are included *Trichoderma* versatile strains (López-Bucio et al., 2015) and plant growth promoting rhizobacteria (Ruzzi and Aroca, 2015). We considered that such microbial plant biostimulants represent one of the solutions for the negative impacts associated with the high residues agricultural systems. Plant growth and development stimulation by microbial biostimulants could compensate delay in the early stage of development. Both plant pathogens biocontrol activities, related to direct competition on plant residues niche and systemic activation of the plant defense mechanisms, reduce the risk of soil-born plant pathogens. Enhancement of nutrient bioavailability and increase nutrients uptake balance nitrogen (and other nutrients) temporary immobilization, resulted from higher carbon inputs into soils.

One of the main constraints for practical use in agricultural systems of microbial plant biostimulants is the development of a bioproduct compatible with the existing application technologies and equipment (Bashan et al., 2014; Berg, 2009; Herrmann and

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Lesueur, 2013). Our first goal on this work was to develop an improved hydro-gelified and film forming formulation, useful for *in situ* formation of a bio-composite mulch, after crop residue treatments. Into this hydro-gelified and film forming composition we tested the inclusion of our microbial biostimulants strain, *Trichoderma asperellum* T36 (Raut et al., 2015) and *Brevibacillus parabrevis* B50 (Oancea et al., 2014).

Our final aim on this work was to evaluate the effect of such crop residue treatment, with microbial biostimulants incorporated into such hydro-gelified formulation, on crop yield and soil characteristics (water stable aggregates and glomalin related soil proteins) related to biological aerated soil structure.

MATERIALS AND METHODS:

Microbial strains. On this study we used two microbial biostimulant strains, *Trichoderma asperellum* T36 NCAIM F 001434 (Raut et al., 2015) and *Brevibacillus parabrevis* B50 NCAIM B 001413 (Oancea et al., 2014), from INCDCP-ICECHIM culture collection. These strains were selected by screening from natural isolates, based on their ability to degrade lignocellulose material and to produce compounds, including volatiles, which inhibits fungal plant pathogens development and stimulate seedling growth (Oancea et al., 2014; Raut et al., 2014). We cultivated these strains on agitated and aerated potato-dextrose broth. After 48 hours and 120 hours of cultivation we harvested the biomass of bacterial and, respectively, fungal biostimulant strain, and we spray-dried the resulted biomass in presence of protectants (Oancea and Dinu, 2010), using a Mini Spray Dryer B-290 (Büchi Labortechnik, Flawil, Switzerland).

Plant material and experimental site. We used hairy vetch (*Vicia villosa* cv. Hungvillosa) as a cover crop and soybean (*Glycine max* cv. Danubian) as the main crop. Hairy vetch was seeded into corn stubble on 21st September 2015, on a seeding rate of 120...125 seed.m^{-2}, corresponding to 38 kg. ha^{-1}. Hairy vetch cover crops were killed by rolling with a roller-crimper and concomitant application of glyphosate (N-(phosphonomythyl) glycine) at 1.1 kg a.i. ha^{-1}, 5 days before establishing main crop. Soybean was sown on April 28th 2015, with a target plant density of 48 plants.m^{-2}, for a seeding rate of 110 kg. ha^{-1}. On all experimental treatments plots soybean seeds were drilled at 3 cm depth into soil, through plant residues mulch, by a pneumatic direct planting machine. The experiment was done on the conservation agriculture platform of NARDI Fundulea, Călăraşi county, Romania, located at 44°27'45" N latitude, 26°31'35" E longitude and 68 m altitude. The averages values of multi-annual temperature, wind speed, sunshine daily duration and total precipitations for this experimental site are: 10.7°C, 3.5 m. s^{-1}, 6.8 h and, respectively, 578 mm. Soil on the experimental site is a cambic chernozem, developed on a loess parental rock, with a dusty - argillaceous Ap 0-27 cm horizon, with 36.5% clay. The soil contains very good levels of potassium (soluble K=175 ppm), phosphorus (70 ppm), and humus (2.2). Total nitrogen is around 0.194 and pH 6.7 (Cociu and Cizmas, 2013). The climatic conditions for 2015 period of soybean vegetation were characterized by higher monthly temperatures (+1.3°C in May; +0.4°C in June; +2.7°C in July; +1.7°C) and lower monthly precipitations (-31.5 mm in May; -22.7 mm in June; -34,9 mm in July) than the average multi-annual. In August the monthly precipitation was higher than average multi-annual, +44.2 mm rain precipitation.

Hydro-gelified and film forming formulation. Our hydro-gelified and film forming formulation, which we used for *in situ* formation of a bio-composite mulch, included following components: (*i*) starch – polyacrylate co-polymer super-adsorbent hydrogels, which retain large quantities of water, slowly releasing it to plants and inoculated plant biostimulants when the water activity into mulch and/or soil decreases; (*ii*) granular wood mulch, based on wood lignocellulose shredded fiber, and intended to increase mulch mechanical stability (and consecutive weed control), and mulch buffering activity on soil temperature and soil moisture; (*iii*) a tackifier based on polyacrylate – polyacrylamide copolymer, which binds together granular and crop residue mulch, improve water retention, improve mechanical stability of the bio-composite mulch and lubricate the application equipment; (*iv*) film forming adhesives, polyvinyl acetate, polyvinyl alcohol, guar gum and corn starch, which further bind mulch ingredients and promote a film formation on soil surface; (*v*) surfactants, cocamidopropylamine oxide and ethylated rapeseed fatty acids, which stabilize the suspension and assure an uniform application of hydro-gelified and film forming composition. We mixed the above mentioned component *in ratio* of 12: 50: 8: 25: 5, respectively. Into this powder mix we homogenized the spray-dried microbial biostimulant biomass, by using a Mini-Glatt fluidized bed processor (Glatt, Binzen, Germany), till a concentration of 10^9 cfu. g^{-1}.

Release of the polyamines and ortosilicic acid from hairy vetch and corn residues. We dried at 45°C hairy vetch and corn stems and leaves and we milled these residues through 1 mm screen. We mixed the ground residues of hairy vetch and corn residues in a ratio of 1: 1 and we sprayed 100 g of the mixed residues with 5 ml 1% hydro-gelified and film forming formulation. Form the resulted treated powder we took 1 g and we suspended in 100 ml of pure water (Milli-Q® Integral, Merck-Millipore, Darmstadt), on a 500 ml Erlenmeyer flasks, covered with cotton wool. We incubated the pure water suspended treated powder on a rotary shaker, at 25°C and 5 rpm, for three weeks. Each two days we sampled 1 ml from the supernatant and we analyzed the released soluble silicon, as ortosilicic acid, using silicomolybdic acid spectrophotometric method (Coradin et al., 2004), with a Merck kit (Merck Silicate Assay, 1.14794, Merck-Millipore), and the released polyamines, with a HPLC method (Taibi et al., 2000). We used as control not treated mixed grounded residues powder.

Field experimental treatments. The soil was fertilized with 60 kg ha^{-1} P fertilizer, applied 5 days before sowing soybean. A spraying volume equivalent to 200 liters per ha, with a mixture of 1.5 l. ha^{-1} commercial herbicide containing 40 g. l^{-1} quizalofop-P-tefuryl and 0.75 l. ha^{-1} commercial herbicide containing 40 g.l^{-1} imazamox was applied as an early post-emergent treatment on all plots. The hydro-gelified and film forming formulation was applied on crop residues, one week after sowing, from 40 cm high, on a dose equivalent to 5 kg. ha^{-1}, dispersed into equivalent spraying volume of 500 l. ha^{-1}, using a backpack sprayer SG20 (Stihl AG, Waiblingen, Germany), with pressure set-up to 275 kPa, and a nozzle with flat jet and low drift (TeeJett® flat-fan TT11002 model, Spraying Systems, Wheaton, IL, US). We organized the experiment in a Latin square design, including 4 treatments in 4 repetitions: V_1 – conservation management, no cover crop; V_2 - conservation management, hairy cover crop mulch; V_3 - conservation management, cover crop mulch treated with hydro-gelified and film forming formulation containing 10^8 spores.ml^{-1} *B. parabrevis* B50 NCAIM B 001413; V_4 - conservation management, cover crop mulch treated with hydro-gelified and film forming formulation containing 10^8 spores.ml^{-1} *T. asperellum* T36 NCAIM F 001434. Each block of a repetition consisted of a plot of 5 m wide and 10 m long.

Soil sampling. We collected soil samples before soybean harvest, on the middle of September, after a rain event which ensured uniform water content. Bulk soil used for aggregate analyses was collected from the 0 to 5 cm and 5 to 10 cm depth of non-wheel traffic inter-rows by compositing four, shallow excavations (0.2 by 0.1 m by 0.01 m deep) from each plot. We took two soil (sub)samples per plot to 30-cm depth from the shallow excavation, with a hand auger sampler (Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands) and we used these (sub)samples for soil glomalin assay. The cores of soil resulted from sampler were cut to obtain 10 to 20, and 20 to 30 cm subsamples. All soil samples were stored in zip lock plastic bags and kept on 4ºC till analyses were performed.

Water stable aggregate assay. We measured water-stable aggregates by using the wet sieving on stacked sieves method (Wright and Upadhyaya, 1998). First, we passed the dried soil samples through a 6 mm screen and then through 2 mm screen. Five grams of soil passed through 2 mm screen were placed on a 1000 µm sieve. Stacked under the 1000 µm sieve were others sieves, a 250, a 125, and a 53 µm, spaced about 1 cm vertically. The sieves set was immersed in pure water (Milli-Q® Integral, Merck-Millipore) until the soil sample was completely covered, then immediately sieved for 3 min at 20 cycles per minute. The length of stroke was 1.3 cm. This stroke and duration were sufficient to clear the screens of slaked soil, leaving only separated aggregates too large to pass through each sieve. The weight of soil retained on each sieve was determined after drying at 40ºC. The proportion of water-stable aggregates (WSA) in each size fraction (WSA_i) was calculated from Eq. (1):

$$WSA_i = \frac{Agg_i - Sand_i}{Soil/1 + Moist - \sum Sand_i} \quad (1)$$

where i is the i^{th} size fraction, Agg is the oven-dry mass of water-stable aggregates collected on each sieve, Sand is the oven-dry mass of sand collected on each sieve, Soil is the oven-dry mass total for 2- to 6-mm aggregates sieved, and Moist is the gravimetric moisture content.

The MWD of aggregates was calculated from Eq. (2):

$$MWD = \sum X_i WSA_i \quad (2)$$

where i is the i^{th} size fraction, and X is the mean diameter of each size fraction, based on the mean inter-sieve size. Water stable aggregate (WSA) is the sand-free, water-stable aggregate mass. Sand is the oven-dry mass of sand collected on each sieve.

Glomalin-related soil protein. We used a procedure for glomalin-related soil protein (GRSP), easy extractable and total, adapted by us (Sesan et al., 2010), from the initially described method (Wright and Upadhyaya, 1998). The procedure was done for 1 g of soil sample. Each sample was initially extracted by autoclaving, at 121ºC for 30 min, of a mixture of 1 g of soil and 8 ml sodium citrate buffer 20 mM, pH 7.0. After cooling and centrifugation for 10 min at 10,000*g* was separated the supernatant (containing easy extractable GRSP) and a residue. The residue was extracted with NaOH for 1 h, at room temperature. The supernatant was removed by centrifugation at 10,000*g* and on remaining residue two additional sequential 1 h extractions were performed, using each time 8 ml of 100 mM sodium pyrophosphate, pH 9.0 and autoclaving at 121ºC for 1 h. The supernatants separated by centrifugation at 10,000*g* from each extraction cycle were combined and the resulting final volume (containing recalcitrant GRSP) was measured. Proteins in the supernatants were analyzed using a Bradford protein assay with bovine serum albumin as standard. Total glomalin / GRSP was calculated as the sum of easy extractable + recalcitrant.

Yield determination. The experimental plots were manually harvested on 17 September 2015. We determined plant height at harvest (cm), number of inserts with pods per plant, number of seeds per pods and total yield (kg. ha^{-1}).

Statistical analysis. The results were expressed as mean of 3 values. Analysis of variance (ANOVA), two ways with replications, and Tukey's test at $p \leq 0.05$ was used to establish the statistical relevance of the results, significant differences being considered at values of $p<0.05$. The Excel software (Office 365 - Excel 2016, Microsoft, Redmont, WA, USA) was used to make calculations and to draw figures.

RESULTS AND DISCUSSION:

We tested the ability of the plant biostimulants microbial strains, incorporated into hydro-gelified and film forming formulation, for their ability to release polyamines and ortosilicic acid from a grounded mixture of hairy vetch and corn residues, on controlled conditions. We selected our plant biostimulant strains, *T. asperellum* T36 NCAIM F 001434 (Raut et al., 2015) and *B. parabrevis* B50 NCAIM B 001413 (Oancea et al., 2014), based on their lignocellulose degradation ability and their capacity to produce compounds, including volatiles, which inhibits fungal plant pathogens growth and development and stimulate seedling growth. We included the spray-dried biomass of these microbial plant biostimulants into the hydro-gelified and film forming formulation. The plant biostimulants microorganisms concentrations into such formulations were reaching 10^9 cfu. g^{-1}. In fig. 1 we present the dynamic of releasing soluble silicon (as ortosilicic acid) and total polyamines (sum of cadaverine, putrescine, spermidine and spermine), into pure water, from the treated mixture of hairy vetch and corn residues. Treatment with our formulated plant biostimulant strains significantly increased the release of soluble silicon and polyamines. Silicon (as ortosilicic acid, non-dissociated on soil pH, up-taken by plant roots) is a plant biostimulant (Savvas and Ntatsi, 2015), influencing nutrients uptake and nutrient use efficiency, delaying plant senescence and alleviating abiotic and biotic stress effects on plants, due to a broad spectrum activation of plant defense system (Van Bockhaven et al., 2013). Polyamines are ubiquitous endo- and exo-signals, involved into plant growth and development (Kusano et al., 2008), plant response to biotic (Jimenez-Bremont et al., 2014) and abiotic stress (Marco et al., 2011), and interactions between plants and beneficial microorganisms (Perrig et al., 2007; Xie et al., 2014). Thus our microbial plant biostimulants, applied into hairy vetch and corn residues, should exert their plant biostimulants effect not only directly, due to their metabolites which influence cultivated plant physiology, but also as an indirect effect, resulted from the active compounds with stimulating effect on cultivated plants, released from treated crop residues laying down on soil surface.

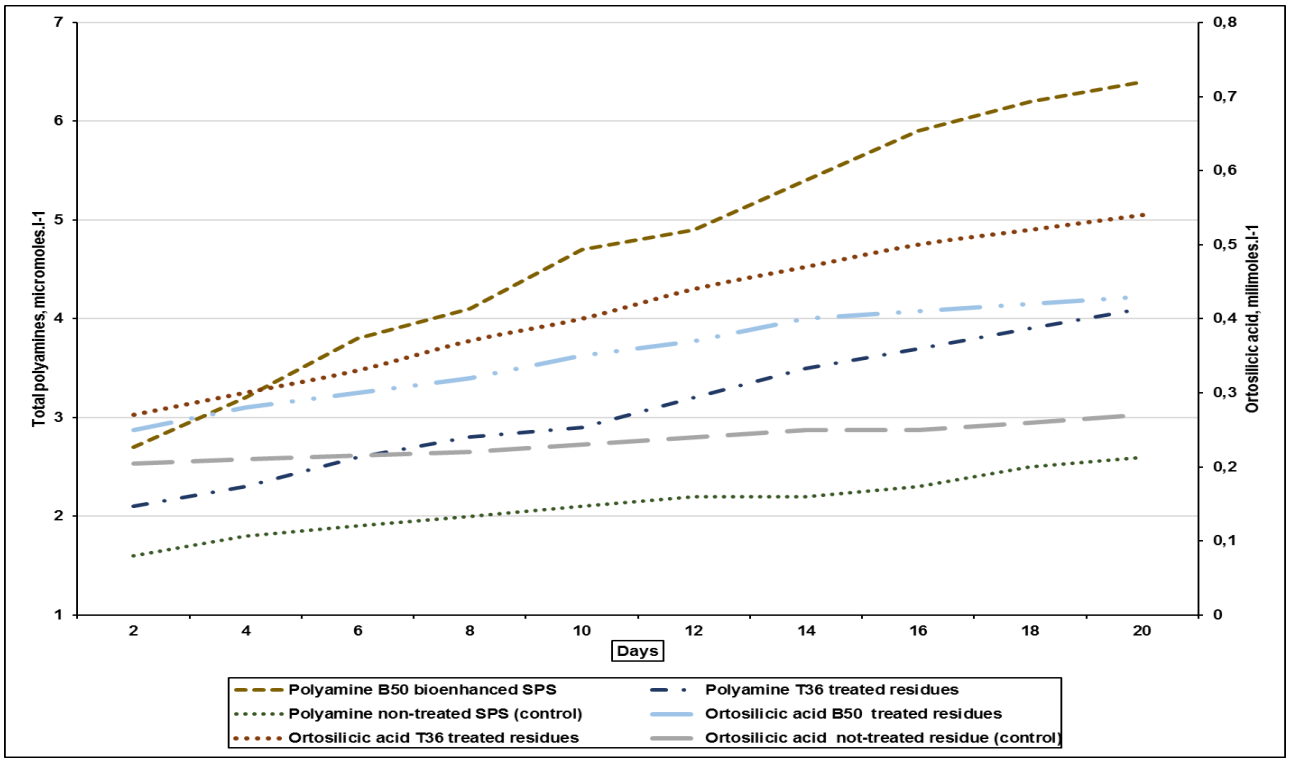

Fig. 1. Dynamic of soluble silicon (as ortosilicic acid) and total polyamines (sum of cadaverine, putrescine, spermidine and spermine), released into pure water from the mixture of hairy vetch and corn residues treated with hydro-gelified and film forming formulation

There is a difference between release of the plant biostimulants compounds, ortosilicic acid and polyamines, from the mixture of hairy vetch and corn residues, between the treatment with the two selected and formulated microbial biostimulants strains. *T. asperellum* T36 release more ortosilicic acid than *B. parabrevis* B50. This is due to the well-known ability of *Trichoderma* strains to produce more efficient extracellular enzymes acting on destructuration lignocellulose matrix (Dashtban et al., 2009) and creating conditions for releasing also biogenic silicon embedded into this matrix (He et al., 2015). *B. parabrevis* B50 produce more polyamines from plant residues, the ability of plant biostimulants gram-positive, spore forming bacterial strains to produce polyamines being a characteristic related to their biological activity (Sicuia et al., 2012; Xie et al., 2014).

The increase of the mean weight diameter is present on both depths of hairy vetch mulch and formulated microbial biostimulants treatments. The use of the formulated plant biostimulants microorganisms as treatment of crop residues slightly increase water stable aggregates compared with the addition of hairy vetch mulch alone. The treatment with *T. asperellum* T36 plant biostimulants microorganisms is influencing at the limit of statistical significance the mean weight diameter of water stable aggregates on the top soil, comparing with not-treated hairy vetch mulch addition to the corn residues, and significantly influence the stability of those from 5-10 cm horizon. Soil water aggregate stability is the result of complex interactions among biological, chemical, and physical processes in the soil (Marquez et al., 2004) This aggregate stability depends on the forces which bind particles together and the nature and magnitude of the disruptive forces. Aggregate stability declines rapidly in soil planted to a clean tilled and maintained crop, ploughing and mechanical weeding generating disrupting force for soil aggregate (Bronick and Lal, 2005). Also clean soil expose top soil aggregate to various disruptive atmospheric forces / stresses - e.g. rain splash (Marzen et al., 2015). Plant residues covering the soil promote aggregate stability because is offering a protection from raindrop impact and cycles of freezing–thawing and drying–wetting (Wuest, 2007). On control, wherein only the corn residues are covering the soil, the mean weight diameter of water stable aggregates is smaller on 0-5 cm depth and 5-10 cm depth (Figure 2), which suggest that the soil coverage with corn residues is not assuring a fully physical protection of the upper horizon from soil, which is reflected on aggregate stability. On hairy mulch treatments, including with formulated plant biostimulants, the mean weight diameters of water stable aggregate are statistically similar for both depths analyzed (0-5 cm, 5-10 cm) and this is showing the existence of similar condition on upper soil horizons.

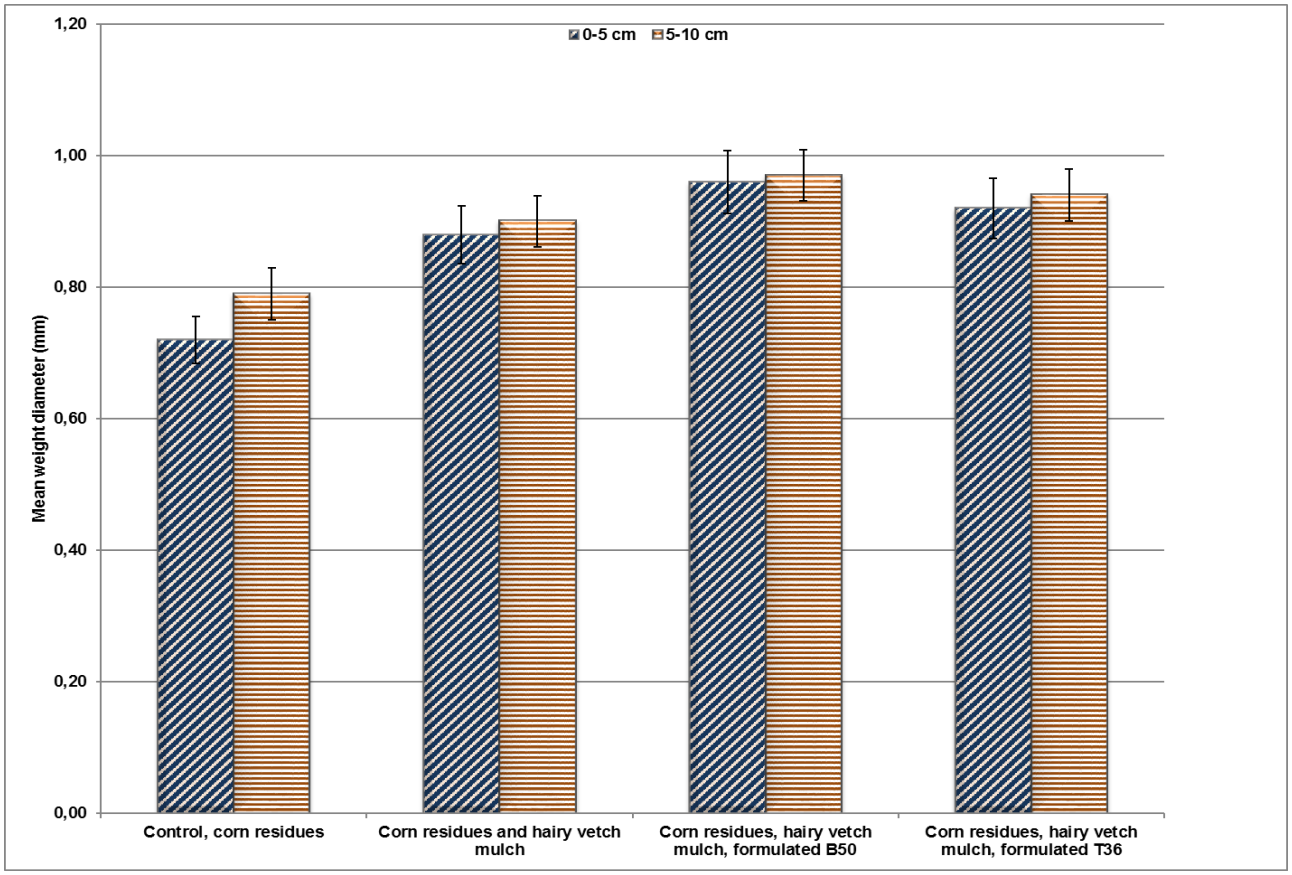

Fig. 2. Mean weight diameter of water stable aggregates in the experimental treatment. Least-square means from mixed model with error bars showing the standard error of each mean.

This improved conditions for aggregate formation is resulting from an improved mechanical protection due to additional coverage provided by hairy vetch over crop during the winter and by additional plant residues during vegetation of the main crop. Application of the film forming formulation (including microbial plant biostimulants) increases mechanical stability of plant residues coverage, protecting supplementary the soil aggregates against atmospheric disruptive forces. Another explanation for increase of water stable aggregates, beside physical protection from disruptive forces, is the enhancement of soil microbial activity by the plant residue coverage; such coverage is buffering atmospheric variation on the soil surface, offering more stable conditions. Beside that, bioactive compounds released from (treated) plant residues are stimulating development of microorganisms involved into formation of water-stable aggregates of the soil near surface. Arbuscular mycorrhizal fungi (AMF), the most active

group of soil microorganisms on soil aggregation (Wilson et al., 2009), are stimulated by the exogenous polyamines (Wu et al., 2012). Thus, the release of polyamine from the plant residues treated with microbial biostimulants strains, could contribute to the formation of water stable soil aggregates. AM fungi were involved into the production of glomalin related soil proteins (GRSP), which has been linked to soil aggregate stability (Rillig and Mummey, 2006; Singh et al., 2013; Wright and Upadhyaya, 1998). Our investigation targeted also on the effect of bioactive mulch on soil GRSP.

The results of analysis of glomalin on different depths and treatment are presented in Table 1. The increase in glomalin-related soil proteins (GRSP) concentrations on hairy vetch mulched treatments (and especially on hairy vetch mulched and treated with formulation of microbial biostimulants) reflects an increase in AMF activity. The easily extractable and total glomalin contents were quite low, but similar to those reported in other studies carried out in soils containing high clay level (Nichols and Wright, 2005; Xu et al., 2015).

Table 1.

Easy extractable (E) and total (T) glomalin-related soil protein (GRSP) concentrations (mg g^{-1} soil) on different soil depths and treatments[a].

Treatment	Depth of soil sample							
	0-5 cm		5-10 cm		10-20 cm		20-30 cm	
	T-GRSP	E-GRSP	T-GRSP	E-GRSP	T-GRSP	E-GRSP	T-GRSP	E-GRSP
Control, corn residues	3.94c	2.02b	4.20c	1.94b	3.58b	1.42b	2.64b	1.32b
Corn residues and hairy vetch mulch	4.61b	2.68a	4.92ab	2.52a	3.46b	1.76a	3.16a	1.42a
Corn residues, hairy vetch mulch, formulated B50	5.53a	2.83a	5.38a	2.58a	3.92a	1.82a	3.08a	1.49a
Corn residues, hairy vetch mulch, formulated T36	5.41a	2.56a	5.06ab	2.56a	3.84a	1.72a	3.22a	1.54a

[a]- Value followed by the same letter do not differ significantly at the P < 0.05 level.

Here we should consider also the humification potential of plant residues, which could lead to a faster complexation of GRSP with the polyphenolics matrix of humic acid (Turmel et al., 2015). Our results presented in table 1 and figure 2 confirm the relationship between GRSP and soil aggregate water stability (Rillig and Mummey, 2006). Treatment with formulated microbial plant biostimulants, proposed in order to counter act the negative side effects of high residues presence on soil, do not reduce the AMF activity and GRSP production into soil. Our results shown that the application of such microorganisms increased water stable aggregates and GRSP (total and easily extractable) on soil. This demonstrate that our microbial plant biostimulant, despite their antagonistic activity toward others microorganisms, stimulate AMF activity. In the case of plant beneficial gram positive, spore-forming bacteria, stimulation of AMF activity was generally reported (Barnawal et al., 2013; Budi et al., 1999; Medina et al., 2003; Vivas et al., 2003). However, contrasting results were reported regarding interaction between AMF and antagonistic / plant biostimulant *Trichoderma*. Several strains of plant beneficial *Trichoderma* were reported to shown antagonism / mycoparasitism against AMF (De Jaeger et al., 2010; Lace et al., 2015; Rousseau et al., 1996). Other studies reported positive results of co-inoculation AMF – *Trichoderma* (Arriagada et al., 2012; Colla et al., 2015; Srinath et al., 2003). Direct interaction is necessary for *Trichoderma* mycoparasitism (De Jaeger et al., 2011; Lace et al., 2015). In our work, application of the hydro-gelified and film forming formulation on the plant residues, leading to a formation of bio-composite mulch, separate *Trichoderma* plant biostimulant strains from AMF, their interaction being mediated through metabolites.

The results regarding the yield proved that application of our formulated microbial plant biostimulants increased the yield of the main crop, soybean – table 2.

Table 2.

Effects of experimental treatments on soybean yield NARDI Fundulea, 2015, non-irrigated

Experimental treatment	Harvest moisture (%)	Average weight of 1,000 seeds (g)	Yield (kg/ ha), normalized 13% humidity	Relative yield	
				% control, corn residues	% corn residue + hairy vetch mulch
Control, corn residues	13.8	192	1421b	100.00%	101.72%
Corn residues and hairy vetch mulch	14.5	202	1397b	98.31%	100.00%
Corn residues, hairy vetch mulch, formulated B50	14.8	212	1564a	110.06%	111.95%
Corn residues, hairy vetch mulch, formulated T36	14.7	208	1535a	108.02%	109.88%
		DL 5%	89 kg/ha	6.2%	

Climatic conditions of the 2015 soybean growing season were difficult, with a significant reduction of monthly precipitation and a significant increase of average temperatures. Our experiment was done on non-irrigated conditions. On such conditions microbial plant biostimulants exerts their effects mainly through activation of the plant response mechanisms to abiotic stress (López-Bucio et al., 2015; Ruzzi and Aroca, 2015). Release of ortosilicic acid and polyamines from plant residues enhance such effects, both plant biostimulants compounds being well-known for their effect on counter-acting drought effect on plants – soluble silicon / ortosilicic acid (Rizwan et al., 2015; Savvas and Ntatsi, 2015; Zhu and Gong, 2013) and polyamines (Farooq et al., 2009; Gill and Tuteja, 2010; Li et al., 2014). Further experiments are necessary to be done, following development of the main crop during early stages, in order to differentiate between various physiological effects of microbial plant biostimulants, direct – i-mediated, produced by the action of their metabolites on plant, and indirect / mediated, by the active biostimulants compounds released from plant residues.

CONCLUSIONS:

The treatment of a grounded mixture of hairy vetch and corn residues with plant biostimulants microorganisms *T. asperellum* T36 and *B. parabrevis* B50, incorporated into a hydro-gelified and film forming formulation, significantly increase the release of polyamines and ortosilicic acid, compounds which are stimulating cultivated plants.

The use of the hydro-gelified and film forming formulation of biostimulant microorganism for treatment of hairy vetch and corn residues increase water stable aggregates for both depths analyzed. This is a result of an increased mechanical stability of plant residues, due to film formation, which assure a better physical protection of top soil against atmospheric disruptive stressors, and of an enhanced activity of soil microbiota, stimulated by the bioactive compounds released from treated plant residues.

Treatment of plant residues with formulated microbial biostimulants increase glomalin-related soil protein (GRSP, Bradford reactive soil protein, total and easily extractable) on soil, due to the enhancement of arbuscular mycorrhizal fungi activity under the influence of polyamines released from hairy vetch and corn residues.

Application of formulated microbial plant biostimulants increased the yield of the main crop, soybean, most probably due to the improvement of cultivated plant response to difficult climatic conditions during 2015 growing season, as a result of both direct biostimulant effects and mediated effects, through ortosilicic acid and polyamines released from plant residues.

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