Call identifier: H2020-SFS-2017-2
BRESOV
Grant agreement N°: 774244

Breeding for Resilient, Efficient and Sustainable Organic Vegetable production

Deliverable No. D5.3

Recommendation for alternative crop fertilization and use of mycorrhiza under water stress conditions

Contractual delivery date:
M52

Actual delivery date:
M52: 31/08/2022

Lead partner:
P18-ITAKA

BRESOV has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No. 774244.

Grant agreement no.	H2020 – 774244
Project full title	BRESOV - Breeding for Resilient, Efficient and Sustainable Organic Vegetable production

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Deliverable title	**Recommendation for alternative crop fertilization and use of mycorrhiza under water stress conditions**
Nature	Report
Dissemination level	PU
Work package number	WP5
Work package leader	P6-FiBL
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Keywords	Alternative fertilisers; microorganisms; organic farming; organic matter; plant-microbe interaction; PGPMs

The research leading to these results has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 774244.

Content

1. Executive summary

The objectives of this task were to provide farmers with recommendations on the use of alternative fertilisers and microorganisms. The trials were carried under on-farm production conditions as well as controlled conditions, in normal water conditions and situation under drought stress.

Our trials did not show significant differences between treatments, in situation of equal total NPK (and different fertilizers) with or without the addition of microorganisms. Working with living material, such as compost, and soils, introduced a great variability especially in terms of microbial composition and organic matter content. The topic showed to be complex and requires extensive trials over several years. To investigate further, we made a bibliographic review on the factors influencing plant-microbe interaction, which was subsequently published.

Further conclusions indicated that well-structured soils, rich in organic mater were less influenced by the introduction of alternative fertilisers and microorganisms. On the contrary, additional trials performed using poorer, less structure soils confirmed a positive effect from microorganisms, and an interaction between microorganisms, betaines and snap bean genotypes, confirming new opportunities to study the interaction between organic matter, microorganisms and plants.

Additionally, we developed a survey on farmers' awareness, use and experience with alternative solutions. The majority of respondents were farmers, with a majority working in organic farming. The questions of the survey were technical and related to what they effectively did on their farms. It showed that most respondents have a good understanding of the technical aspects, use alternative fertilisers as well as microorganisms and in general recommend their use.

2. Introduction

WP5 acts as a bridge between breeders and farmers and provides new material with promising attributes into the field on organic farms to be tested under regular organic production methods. This deliverable presents the results of Task 5.3 on alternative fertilizers and microorganism formulations. In this task, we assessed new products to help cope with drought stress (increasing Water Use Efficiency), and products to increase Nitrogen use efficiency (NUE) and subsequently crop performance.

The results were obtained from multiple sites where we tested how to improve crop performance by acting on soil activity and plant absorption. Three basic ingredients were used: compost to provide nutrients, a commercial formulation containing microorganisms to promote soil vitality together with amino acid to provide organic nitrogen to microorganisms, and organic fertilisers to complement fertilisation programmes.

The objectives were to provide farmers with practical recommendations on how to effectively use alternative crop fertilisation solutions such as compost and microorganisms to improve production.

3. Description of Activities

In the first part of the Task (Part 1 Crop Performance), UNICT, SECL and FiBL performed experiments using local compost to fertilize the crops and formulations of bacteria and amino acids provided by ITAKA. In these tests, plants from a commercial organic variety from each crop was grown in one site in Sicily (UNICT), one site in France (SECL) and one in Switzerland (FiBL) and treated with: Compost, ITAKA's formulations of bacteria and amino acids, and a standard organic fertilizer suitable for each crop. Measurements included yield, overall quality and Nitrogen acquisition by the plants and in the soil.

Original program included the following trials:

- 2019: SECL Tomato, FiBL Tomato
- 2020: SECL Broccoli, SECL Beans, UNICT Tomato

In the second part of the task (Part 2 Water Stress) on the same sites, experiments were planned to investigate the growth response of tomato and bean plants when inoculated with mycorrhizal fungi, and the potential effect of the mycorrhizal association on drought tolerance

The following programme was planned:

- 2019: FiBL Beans, FiBL Tomato
- 2020: SECL Tomato, FiBL Tomato

4. Results

The following protocol was defined in order to compare a standard situation with or without the addition of organic matter, microorganisms + amino acid or both:

Treatment 1 – Control plot: Local standard fertilization practice. For SECL this would consist of a local vegetal compost mixed with manure. However, as the control needs to be the same for all, we have agreed to use a commercial organic fertilizer, referred here as Standard NPK Fertilisation.

Treatment 2: Standard NPK fertilization (as per treatment 1) + Itaka's microorganism formulation "XP191BS" at the dose of 2kg/ m^3 + ammino acid*.

Treatment 3: Local compost from municipal green waste, possibly with manure. FiBL and SECL sharedthe properties of their usual or best compost candidates and agree on the compost with the most similar properties (i.e., comparable local composts)- completed with the standard fertilizer to obtain same total available NPK between all treatments of each location.

Treatment 4: Compost (as per treatment 2) + Itaka's formulation of microorganisms "XP191BS" + ammino acid* (as per treatment 3) – completed with the standard fertilizer to obtain same total available NPK in all treatments.

** SECL used the amnino acid provided by ITAKA, while FiBL used a liquid N fertilizer at 9% of similar characteristics.*

All treatments had the same level of available elements N-P-K-Ca. The level was decided for each of the 3 crops on account of production requirements and was applied to all tests.

The product XP191BS is an inoculant preparation containing mycorrhizal fungi and rhizosphere microorganisms, used in situation of soil fatigue or damaged by excessive farming. It has the following composition:

COMPOSITION	Concentration
Bacillus spp. (B. subtilis; B. amylofiquefaciens; B.megaterium)	≥ $1x10^9$ CFU/g
Pseudomonas spp. {lurida e fluorescens}	≥ $1x10^8$ CFU/g
Streptomyces spp. (S. griseus ; S. lydicus)	≥ $1x10^7$ CFU/g
Trichoderma spp (T. harzianum, T. asperelum,T. atroviride)	≥ $1x10^6$ CFU/g
Bacillus pumilus	≥ $1x10^3$ CFU/g
Bacillus azotoformans	≥ $1x10^3$ CFU/g
Saccharomyces cerevisiae	≥ $1x10^3$ CFU/g
Mycorrhizae (Glomus mosseae and intraradices)	≥ 1%

The ammino acid product used by SECL is a commercial product named AMMINO COMPLEX EXTRA, defined as a Fluid hydrolysate fertilizer containing Nitrogen (N=8%), Boron (B), Copper (Co), Iron (Fe), Manganese (Mn), Zinc (Zn).

FiBL use a commercial product “BIORGA N Flussig” (N=9%).

4.1. Crop performance:

In this part, four trials provided useful results, three on tomato, one on broccoli, and a trial on beans was abandoned for unfavourable weather conditions:

4.1.1. SECL test on tomato greenhouse GR – March 2019:

Results:

The assessment made at the first harvest showed very similar results in plant development. Only Treatment 2 showed a slight positive difference on the N° of flowers, stem diameter and leaf length, although non-significant.

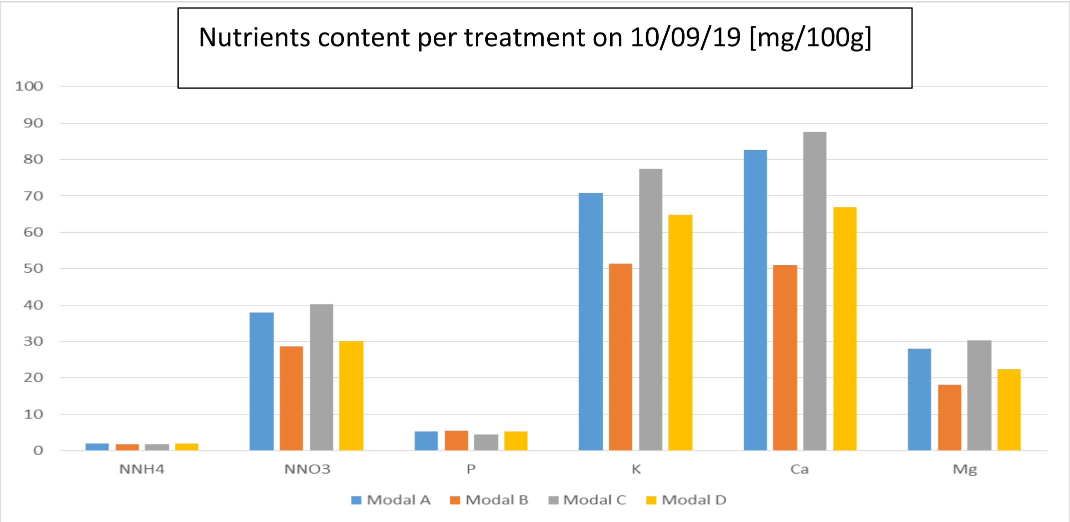
Assessment 16/05/2019 pre-harvest	**Plant Stage**	**N° flowers**	**Stem diameter**	**leaf length**
Treatment 1: Modality A = NPK only	7,95	3,20	9,08	37,93
Treatment 2: Modality B = NPK only+ XP191BS	7,90	3,50	9,60	39,75
Treatment 3: Modality C = NPK + compost DV 32.5 T/Ha	7,90	3,00	8,40	37,98
Treatment 4: Modality D = NPK + compost DV 32.5 T/Ha + XP191BS	7,90	3,00	8,40	37,98

There were no significant differences for yield. XP191BS seemed to have a slight depressive effect, which could not be explained.

	Yield brut kg/m²	Yield net kg/m²	Degrade en g/m²	AMW en g	% cat extra	number of grapp/m²	% waste
Modality A	35.3	**34.9**	236	**132**	85	**46.6**	1.1
Modality B	33.4	**33.0**	230	**128**	85	**44.9**	1.0
Modality C	33.9	**33.4**	247	**131**	86	**44.6**	1.5
Modality D	33.2	**32.8**	320	**129**	85	**44.8**	1.2
P value	-	***0.30***	-	***0.37***	*0.99*	***0.24***	-
Moy AC = without Maxy soil	34.6	**34.1**	241	**132**	85	**45.6**	1.3
Moy BD =with Maxy soil	33.3	**32.9**	275	**129**	85	**44.8**	1.1
P value		***0.29***		***0.97***	*0.79*	***0.18***	-
Moy AB = without compost	34.3	**34.0**	233	**130**	85	**45.7**	1.0
Moy CD = with compost	33.5	**33.1**	284	**130**	86	**44.7**	1.4
P value	-	***0.15***		***0.09***	*0.97*	***0.31***	-

Modality = treatment, Moy. = Average

Soil analysis performed at the end of the trial show limited but constant difference in elements such as for N from NO3, k, Ca and Mg. This did not correlate with any effect on plants development, may be due to an increased consumption by the microorganisms, but not affecting positively the plant, and on the contrary showing signs of depressing the plant.

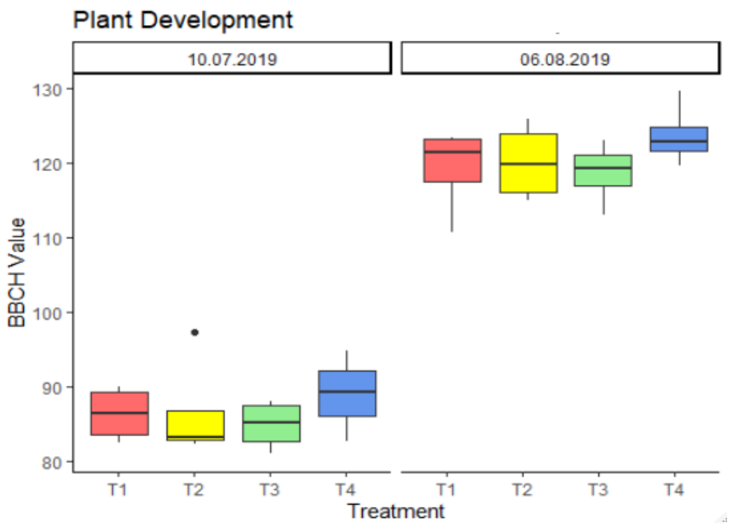


This trial did not establish any significant effect from XP191BS, except a limited impact on vegetation in Treatment 2, without any direct effect on yield.

4.1.2. FiBL test on tomato greenhouse GR – April 2019:

Results:

Results confirmed no significant differences in plant development, although in Treatment 4 a faster plant development was observed (not significant).



A data analysis showed no significant impact of the different fertilisation and inoculation treatments on yield and yield related variables (Figure A, B, C below). Treatment 4 (NPK+Compost+XP191BS) shows the highest total yield (132.5±9.4 kg). Treatment 3 and 4 show the highest yield per plant (13.2±1.4 and 13.2±1.0 kg) and highest average tomato weight (98.6±2.0 and 100.1±0.9 g). Differences between treatments were not significant.

Figure A: Total yield

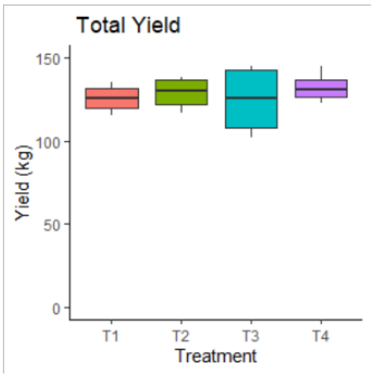


Figure B: Yield per plant

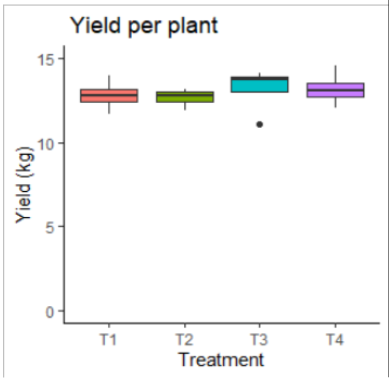
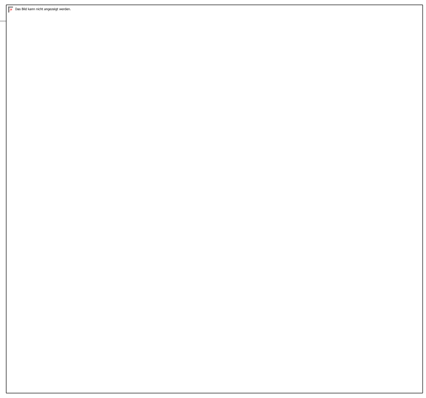
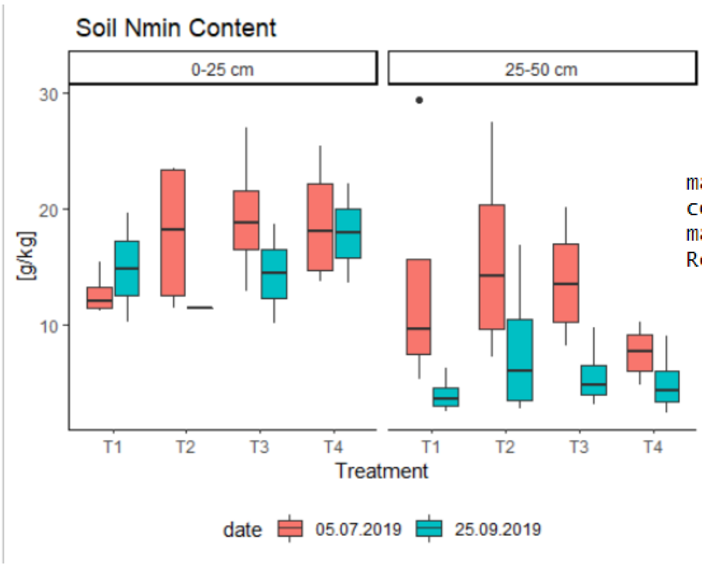


Figure C: Average fruit weight



The $N_{mineralized}$ (N_{min}) analysis confirmed that the N content was not significantly different among treatment at the beginning of the test. At the end of the test, the N present was very low at the 25-50 cm depth, but no significant differences could be found.



	Df	Sum Sq	Mean Sq	F value	Pr(>F)
maxi	1	0.05	0.0451	0.041	0.840
com	1	0.01	0.0057	0.005	0.943
maxi:com	1	1.39	1.3889	1.270	0.265
Residuals	53	57.98	1.0940		

4.1.3. SECL test on broccoli open field OP – April 2020:

Results:

Growing conditions were difficult and stressful during the post-transplant period and during the period of inflorescence emergence. This resulted in significant plant loss of (-28 to -19%) and downgrading to second grade 79 to 89% of cabbages harvested.

Treatments	Total yield (t/ha)	% Marketable	Marketable yield (t/ha)	% 1st grade
T1	7,45	62%	4,61	11%
T2	7,32	59%	4,31	11%
T3	8,14	66%	5,35	16%
T4	7,27	76%	5,52	21%

The use of compost and XP191 BS did not alleviate this phenomenon.

4.1.4. UNICT test on tomato open field – June 2020:

This test was organized on the basis of a different protocol, to study the relationship between Organic Matter content and crop performance using natural bioactive formulation of microorganisms (XP191BS). Three levels of organic matter were tested and three different cultivars in order to evaluate possible influence of the cultivar on the performance.

1st experimental factor: Soil organic matter percentage
OM25 (25% organic matter and 75% volcanic sand)
OM50 (50% organic matter and 50% volcanic sand)
OM75 (75% organic matter and 25% volcanic sand)

2nd experimental factor: Genotype
A. Rubinek hinired, SEMO.CZ ltd – bush cherry tomatoes
B. Orbit, SEMO.CZ ltd – bush tomato of medium early type with firm, oval fruits
C. Pavlina SEMO.CZ ltd – bush tomato, medium early variety with big semi-firm fruits

Method:

- Sowing date: 10/04/2020;
- Transplanting date: 19/05/2020 One plant per pot (25 cm diameter) placed along two rows
- Soil nutrition before transplanting: Amino acid (AMMINOCOMPLEX EXTRA) + XP191BS

Soil characterisation:

GRANULOMETRY (g kg^{-1})	OM25 BEFORE	OM25 AFTER	OM25 MEAN	OM50 BEFORE	OM50 AFTER	OM50 MEAN	OM75 BEFORE	OM75 AFTER	OM75 MEAN	OPTIMAL RANGE
SKELETON	412	283	347,5	348	313	330,5	412	312	362	
FINE SOIL	588	717	652,5	652	687	669,5	588	688	638	
SAND	923	927	925	939	943	941	923	943	933	(500 – 750)
LOAM	31	34	32,5	14	187	100,5	31	18	24,5	(150 – 400)
CLAY	46	39	42,5	47	39	43	46	39	42,5	(50 – 250)
Chemical parameters										
pH	8,6	8,6	8,6	8,4	8,4	8,4	7,9	8,5	8,2	(5,5 – 7,5)
ORGANIC CARBON (g kg^{-1})	23	25,1	24,05	85	69,8	77,4	203	82,3	142,65	MIN 12
ELECTRIC CONDUCIBILITY (dS m^{-1})	4,71	1,33	3,02	3,19	2,64	2,915	4,02	1,36	2,69	(0,2 – 1,9)
CSC (meq 100g^{-1})	13,4	16	14,7	25,8	15,3	20,55	53,2	36,2	44,7	
TOTAL NYTROGEN (g kg^{-1})	7,6	1,4	1,4	3	3,1	3,1	1,7	4,4	3,9	MIN 1
C/N RATIO	7,8	17,9	12,85	16,5	22,5	43,15	15,5	18,7	17,1	

Results:

The major yield factors were measured. Plant fresh weight was superior in the factor OM75; however, this difference was not observed in the dry weight. The number of trusses was not significantly different. Fruit number and yield confirmed that OM75 was significantly better than OM25 and OM50.

THESIS	G	TRUSSES (n)	FRUIT (n)	FRUIT WEIGHT (g)	FRUIT YIELD (kg m^{-2})
OM25		1.96 ± 0.48 a	7.47 ± 8.24 b	29.21 ± 17.63 b	6.23 ± 0.20 b
OM50		2.03 ± 1.44 a	8.38 ± 9.85 b	45.39 ± 32.66 ab	8.88 ± 0.25 b
OM75		2.39 a ± 1.46 a	23.80 ± 27.57 a	53.01 ± 41.32 a	19.75 ± 0.50 a
	A	2.09 ± 1,78 a	31.52 ± 23.49 a	14.53 ± 10.71 b	16.37 ± 0.45 a
	B	1,96 ± 1.04 a	5.22 ± 3.02 b	55.67 ± 26.96 a	12.15 ± 0.24 a
	C	2.33 ± 1.23 a	2.91 ± 2.77 b	58.64 ± 36.67 a	6.35 ± 0.26 b
SIGNIFICANCE					
OM		ns	***	*	***
G		ns	***	***	**
OM x G		**	***	*	ns

Organic matter content showed to positively influence the effect of nutrition in protocol based on microorganism and amino acids. It also confirmed a significant interaction between organic matter content and genotype.

4.1.5. Discussion on crop performance:

The effect of natural bioactive products and microorganisms on crop performance was not demonstrated in the tests carried out. Conclusions showed no significant interactions between microorganisms and plants, but only positive influence on development for Treatment 2 or fresh weight and yield for Treatment 3 and 4.

In parallel, the test run by UNICT (3.1.4.) showed that Organic Matter content could affect plant performance, confirming that many factors may influence the role of microorganisms and the interactions between microbiome and plants.

By doing field trials in several locations, we faced an agronomical variability, which was expected, but which was not compensated by the introduction of significant quantities of Fresh Organic Matter (FOM). A better characterisation of decomposition mechanisms or possible quantification of soil vitality may have been necessary to define the parameters required to evaluate the potential Growth Promotion activity of microorganisms.

4.2. Water stress:

To study the effect of microorganisms in water stress conditions, FiBL performed several trials on beans and tomatoes in 2019 and 2020 in pots in a first stage to define the methodology needed to perform such water stress trials. Three factors were studied: soil type, inoculation and water stress level. SECL carried trials in production conditions in greenhouse (tomato). In all trials, at least four treatments were applied and consisted of a normal irrigation and a reduced irrigation and for each irrigation type one treatment with the application of microorganism formulation and one without. SECL performed the treatments described in previous section with for each two treatment levels. Additional treatments included by FiBL, were the planting substrate (composition, Organic Matter content) and in one trial, an additional fertilizer.

4.2.1. SECL plants test on tomato greenhouse GH – 2020:

Irrigation regimes: 2 modalities

- CONTROL regime = 100% of calculated ETP (Evapotranspiration Potential).
- REDUCED regime = 70% of calculated ETP

100% ETP means covering 100% of the water losses through irrigation – Business As Usual

The “reduced” regime aims to simulate a water stress situation and to highlight the potential effect of the formulation in this situation.

Results:

Plant development did not differ significantly within each water level. The stressed level produced a significant reduction in plant development in stem and head rod diameters.

	Stem diameter at 2nd bouquet in mm *	Head rod diameter in mm *	Length of leaf in flower bouquet in cm **	Bouquet / head distance In cm ***
Modality T1				
100% ETP	11.5	8.0	32.7	19
70 % ETP	10.9	7.7	34.2	18.2
Modality T2				
100% ETP	11.2	8.3	32.4	19.1
70 % ETP	10	7.6	31.9	19.6
Modality T3				
100% ETP	12.6	9.1	34.7	18.7
70 % ETP	10.4	7.7	33.9	19.6
Modality T4				
100% ETP	11.4	8.1	31.7	19.5
70 % ETP	9.9	7.9	32.7	17.5
Medium T1	11.2	7.9	33.4	18.6
Medium T2	10.6	7.9	32.1	19.4
Medium T3	11.5	8.4	34.3	19.2
MediumT4	10.6	8.0	32.2	18.5
P value	0.48	0.53	0.28	0.96
Medium 100% ETP	11.7 (a)	8.4 (a)	32.9	19.2
Medium 70% ETP	10.3 (b)	7.7 (b)	33.2	18.6
P value9	0.004	*0.041*	0.73	0.7

* the larger the diameter, the more vigorous the plant is considered
** the longer the leaf, the more the plant is considered vegetative
*** the greater the distance between the bouquet and the head, the more vegetative the plant

Modality = Treatment

Results on yield showed a significantly lower production in Treatment 2 (standard NPK fertilization + microorganism formulation + amino acid), in both irrigation levels (see table below). No conclusions could be drawn. Water stress, as expected, influenced yield and N° of clusters.

	Gross yield kg / m²	Net return kg / m²	PMC en g	% cat extra	Number of clusters / m²	BER g/m²	Waste g/m²
Modality T1							
100% ETP	23.0	19.9	119	71	31.6	642	2293
70 % ETP	22.6	20.6	119	76	31.8	270	1459
Modality T2							
100% ETP	16.8	13.1	96	61	26.2	529	2128
70 % ETP	14.7	13.5	117	73	18.7	742	874
Modality T3							
100% ETP	21.8	19.1	114	74	30.4	83	2205
70 % ETP	22.6	20.7	115	76	32.5	342	1503
Modality T4							
100% ETP	22.4	20.1	110	75	33.5	293	1735
70 % ETP	17.4	13.5	104	66	25.2	1142	1796
Medium T1	**22.8 (a)**	**20.3 (a)**	**119**	**74**	**31.7 (a)**	**440**	**1876**
Medium T2	**15.8 (b)**	**13.3 (b)**	**107**	**67**	**22.5 (b)**	**576**	**1501**
Medium T3	**22.2 (a)**	**19.9 (a)**	**114**	**75**	**31.5 (a)**	**223**	**1854**
Medium T4	**19.9 (a)**	**16.8 (a)**	**107**	**71**	**29.3 (a)**	**746**	**1766**
P value	*0.00013*	*0.0005*	*0.18*	*0.47*	0.0027	0.098	0.74
Medium 100% ETP	***21 (a)***	**18.1 (a)**	**110**	**70.5**	**30.4 (a)**	**386 (b)**	**2091 (a**
Medium70% ETP	***19.3 (b)***	**17.1 (b)**	**114**	**72.9**	**27.1 (b)**	**624 (a)**	**1408 (l**
P value	*0.049*	*0.32*	*0.38*	*0.53*	0.045	0.017	0.02

In the same way, the irrigation level impacted root weight measured at the end of the trial. There was no major incidence of the treatments on soil nutrient content.

Element		Laboratory results				
		Mod 1 dry	Mod 2 dry	Mod 3 dry	Mod 4 dry	Average dry
pH		6.97	7.13	6.96	6.82	6.97
Organic carbon (%)		3.9	3	4.3	3.2	3.6
Active organic matter (%)		6.8	5.2	7.4	5.6	6.25
Conductivity at 25°C (mS)		1.57	1.75	1.61	1.45	1.60
N-NH4 (mg/kg)		0.4	0.4	0.5	0.5	0
N-NO3 (mg/kg)		20	23	23	19	21
P2O5 (mg/kg)		23.0	18.0	24.0	20.0	21
K2O (mg/kg)		113	115	121	99	112
CaO (mg/kg)		83	109	83	77	88
MgO (mg/kg)		39	45	42	38	41

The results obtained did not make it possible to demonstrate the effectiveness of the formulation. We even perceived a small depressive effect on agronomic results, which is difficult to explain: is there a root / bacteria competition on nutrition, especially nitrogen? Despite different growing conditions between 2019 and 2020, namely later planting, no-grafted plants vs grafted leading to strong contamination by *Pyrenochaeta lycopersici* in 2020, these results on crop performance were similar between both years. Furthermore, we could not see any notable effect of the microorganism formulation tested in a situation of water restriction.

4.2.2. FiBL pot test on tomato greenhouse GR – 2020:

Protocol:

1st experimental factor: soil

- V1: 90% substrate Dompierre + 10% perlite
- V2: 50% sand + 45% substrate Dompierre + 5% perlite

2nd experimental factor: inoculation

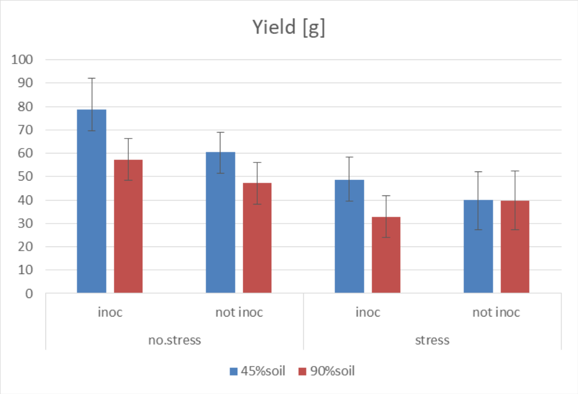
- Untreated
- XP191BS: 2 applications (before transplant, 10 days after transplant), dose 0.2 g/plant per application

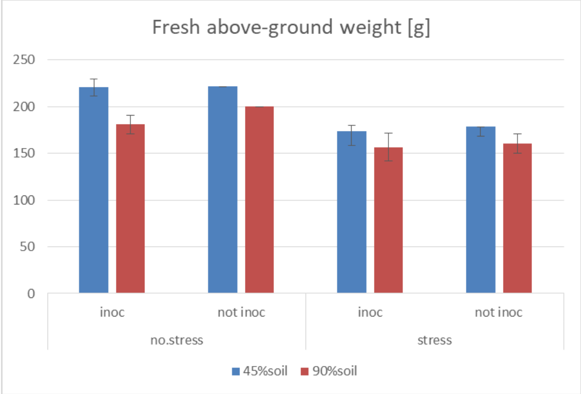
3rd experimental factor: water stress

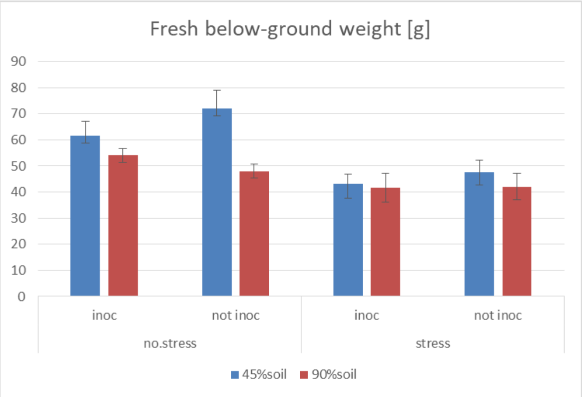
- CONTROL regime = 100%
- REDUCED regime = 75% of control

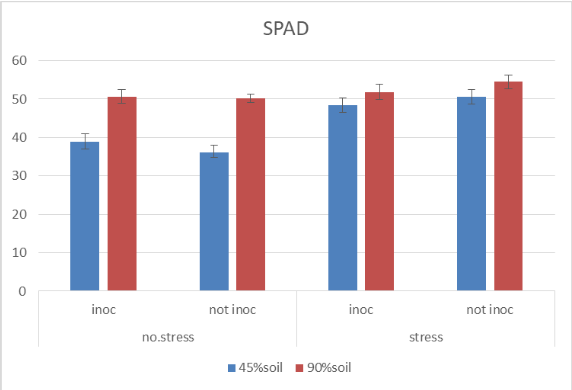
The trial was conducted until the first clusters of fruits at the end of September, when it had to be terminated due to the onset of a russet mite infestation. Data was acquired as planned: regular evaluation of stress and other symptoms (chlorosis, spots, P-deficiency, disease), SPAD measurement, above-grown and below-ground fresh and dry weight, vigour evaluation, root evaluation and yield. Root samples were acquired for microorganism colonisation measurement.

Results:









Results confirmed that differences were due to soil type and water stress. No effect from innoculation with the microorganism formulation was observed.

ANOVA significance:

Yield:	Soil			
Above-ground Biomass:	Soil	Stress		
SPAD:	Block	Soil	Stress	Soil+Stress
P defficiency:	Soil	Stress	Soil+Stress	

Other trials carried to optimize the method including pot trials on beans and trials on the field are not shown. No trial could show a better plant performance under stress when the microorganism formulation was applied.

4.2.3. Discussion on water stress:

Results showed that soil and stress were the main sources of differences. Inoculation did not affect the plants in the control regime nor the stress regime.

Although the number of tests was relatively limited, it became clear that other factors were involved in the process of interaction between soil and microbiome.

Microorganisms such as Trichoderma spp. and Bacillus spp. contained in XP191BS are known to improve in different ways the humification process, using organic nitrogen present to reduce organic fractions. As a consequence of the process, nutrients are made available to the plant and the C/N ratio is reduced. (ref. **Alquati G., Rizzitano G., 1997; Radaelli L., Calamai L., 2001.** Jansson et al, 1982; Whitmore et al, 1997; Clay et al, 1990; Mueller et al, 1998)

The C/N ratio provides a measure of a soil humification level. The standard scale used is the following:

- between 0-9: low, often found in soils with low organic content and poor nutrient availability.
- between 9-11: optimum balance between humification rate and nutrient availability. Humification is stable over time and is an indication of soil vitality.
- above 11: high, corresponding to soils rich in organic matter but potentially facing slow humification due to limited presence of N and possibly N shortage.

In situations where the original C/N ratio and the Soil Organic Matter (SOM) is high, as it was experienced in test 1 Crop Performance (SECL tomato greenhouse) and test 2 Crop Performance (FiBL tomato greenhouse), part of the organic matter is not transformed and the process of humification and mineralization is slow. In this case the microorganisms may have a limited role in the humification process due to the limited presence of Nitrogen.

This same situation repeated itself in test 1 Water Stress and test 2 Water Stress.

Test 3.1.1. Crop Performance: SECL tomato

	Beginning test
C %	2.1
OM %	5.1
C/N	88

Test 3.1.2. Crop Performance: FiBL tomato

	Beginning test
C %	1.74
OM %	3.0
C/N	99

Agronomically, plants may develop normally in situation of high C/N ratio if nutrients are available for the duration of the cycle. In this case, the addition of extra compost (Fresh Organic Matter) or extra microorganisms, as shown in the test 1 and 2 results, may not affect the soil characteristic nor the plant performance including resistance to stress.

In test 3.1.4., where conditions for humification could be considered more adequate, results showed that an increase in organic matter (OM) was correlated with an increase in number of fruits and yield.

Test 3.1.4. Crop Performance: UNICT tomato open field

Beginning test	OM25	OM50	OM75
C %	2.3	8.5	20.3
OM %	3.9	14.7	34.9
C/N	7.8	16.5	15.5

It demonstrated that further information on what influences the effect of microorganisms on the plants is required in order to include additional factors in further experimentation. Combinations of factors may be involved.

5. Deviations

These first results were seen as an opportunity to check whether similar experiments using microorganisms were present in **scientific literature** and if they could explain our situation. A **bibliographic review** of existing in vitro studies was organised to complete our knowledge on:

- The role of Plant Growth-Promoting microorganisms (PGPMs)—especially bacteria and fungi—in increasing the plant's capacity to absorb nutrients and its water use efficiency as well as inducing resistance against plant diseases.
- The factors modulating these interactions such as the environmental conditions, but also the plant genotype and the microbiome already present in the soil.

The aim was to summarize the factors that can influence the beneficial effects of PGPM application and the considerations necessary to maximize their effectiveness.

Additionally, it was agreed that on the basis of the review findings, UNICT would perform extra field trials integrating potential new elements coming out of the review.

We also considered interesting to evaluate the **farmers awareness** on the use of natural, alternative or recycled fertilisers and microorganism formulations in agriculture in various areas in Europe. A **comprehensive survey** was developed between ITAKA and FIBL to understand better the perception farmers have on alternative solutions. The survey was done on LimeSurvey and hosted on the FiBL servers (for Data Protection) in English, French, German, Italian and Spanish, and disseminated through the BRESOV and our respective institutions networks.

5.1. Bibliographic review:

The manuscript was elaborated during 2021 and published by UNICT in Sustainability:

"Plant-Microbe Interaction in Sustainable Agriculture: The Factors That May Influence the Efficacy of PGPM Application." Sustainability 2022, 14, 2253.

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Abstract: The indiscriminate use of chemical fertilizers and pesticides has caused considerable environmental damage over the years. However, the growing demand for food in the coming years and decades requires the use of increasingly productive and efficient agriculture. Several studies carried out in recent years have shown how the application of plant growth-promoting microbes (PGPMs) can be a valid substitute for chemical industry products and represent a valid eco-friendly alternative. However, because 16ft he complexity of interactions created with the numerous biotic and abiotic factors (i.e., environment, soil, interactions between microorganisms, etc.), the different formulates often show variable effects. In this review, we analyze the main factors that influence the effectiveness of PGPM applications and some 16ft he applications that make them a useful tool for agroecological transition. *Keywords: PGPR; PGPF; organic farming; plant-microbe interaction; sustainability; biocontrol*

https://www.mdpi.com/2071-1050/14/4/2253

5.2. Additional trials from UNICT:

In the review, studies showed that the addition of betaine in situation of water stress increases plant tolerance by inducing the synthesis of antioxidants, both enzymatic and non-enzymatic which protect the plant from damage.

On this basis, subsequent trials were run at UNICT looking at the added effect of Betaine and microorganisms on plant development in water stress conditions.

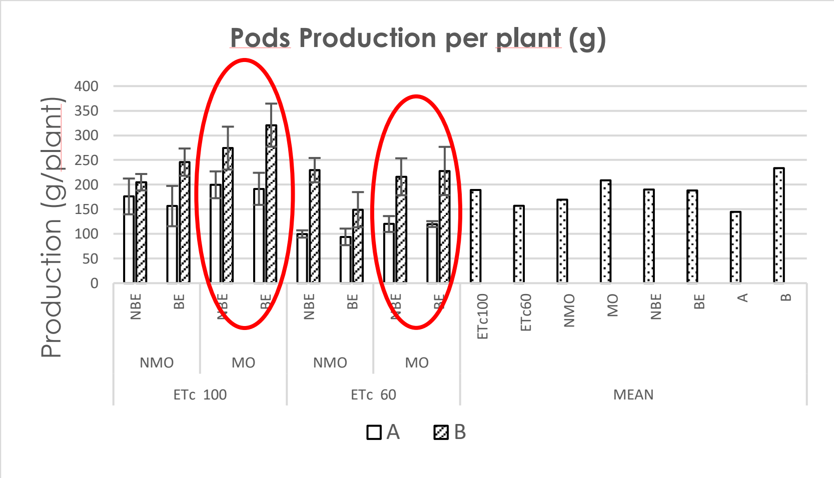
5.2.1. Effects of microbial consortia and betaines on greenhouse snapbeans grown under water stress conditions.

Factors:

1. water requirement based on crop evapotranspiration (ET), two different water requirements: 100% and 60% of ET_c (ET_c 100 and ET_c 60, respectively);
2. Application of microorganisms (MO) using XP191BS;
3. Application of betaines (BEs) using a commercial product;
4. two green beans cultivars (CV): 'Domino' and 'Maxi'.

Results:

The plant epigeous fresh weight varied significantly in relation to ET_c from 153.8 to 200.2 g respectively for ET_c 60 to ET_c 100, to MO from 175.3 to 225.1 g for NMO and MO respectively. Similar differences were observed also for the plant ipogeous fresh weight varied significantly both for ET_c, MO and CV, ranging from 15.2 and 17.1 g for ET_c 60 and ET_c 100 and from 15.1 to 19.0 g for NMO and MO, and finally from 14.2 to 19.9 g for B and A respectively. The plant epigeous dry matter was significantly affected by the interaction ET_c x BE ranging from 16.1 to 63.4% for the A cultivar grown by ET_c 100 x MO x BE and for the same cv grown by ET_c 60 x MO x BE, instead ranging from 15.0 to 46.9% for the B cultivar grown by ET_c 100 x MO x BE and by ET_c 60 x MO x BE. Whereas the plant ipogeous one, by the betaines and the interactions ET_c x MO x BE ranging from 60.8 to 71.9% for ET_c 60 x MO x BE and ETc 100 x MO x BE respectively for the A cultivar and from 58.7 to 67.0% for the B cultivar. Regarding the ramification of 1st order branch, was significantly affected by the interactions ETc x BE and CV x BE with the higher ramification reported for A cultivar by BE applied ranging from 7.3 to 9.0 for ET_c 60 and ET_c 100 respectively, compared to the B cultivar ranging from 5.3 to 5.7 for ET_c 100 and ET_c 60 respectively. Furthermore, the ramification of 1st order branch was affected by the interaction ET_c x MO x BE, higher value was reported without MO application.



One of the two cultivars tested shown more productivity in both in optimal irrigation and water stress condition. The data obtained from the test confirm the efficacy of microbial based treatments based in increasing yields, both alone and in combination with Betaines. Furthermore, the data shows how the application of MO can also improve the quality of the pods, in this case increasing the diameter compared to what is observed on plants to which MO has not been applied. The efficacy of the treatments is variable according to the genotype, probably due to the complexity of the interactions between plant, MO and the environment, which is extensively studied in the scientific literature.

	ET_c	MO	BE	CV	ET_c x MO	ET_c x BE	ET_c x CV	MO x BE	CV x MO	CV x BE
Plant epigeous fresh weight (g)	***	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Plant ipogeous fresh weight (g)	*	*	n.s.	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Epigeous dry matter (%)	***	n.s.	*	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.
Ipogepus dry matter (%)	***	***	***	n.s.	***	n.s.	n.s.	n.s.	n.s.	n.s.
Production of pods per plant (g)	***	***	n.s.	***	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Pod number (n)	n.s.	**	n.s.	***	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Pod weight (g)	***	n.s.	n.s.	***	n.s.	n.s.	***	n.s.	n.s.	n.s.
Pod diameter (mm)	n.s.	**	n.s.	***	*	n.s.	**	n.s.	*	*
1° order branch	n.s.	n.s.	***	***	n.s.	**	n.s.	n.s.	n.s.	**
ET_c = crop evapotraspiration; MO = microorganisms; NMO = No microorganisms; BE = betaines; NBE = No betaines; CV= cultivar;A = cv 'Domino'; B = cv 'Maxi'										

	ET_c x MO x BE	ET_c x MO x CV	ET_c x BE x CV	CV x MO x BE
Plant epigeous fresh weight (g)	n.s.	n.s.	n.s.	n.s.
Plant ipogeous fresh weight (g)	n.s.	n.s.	n.s.	n.s.
Epigeous dry matter (%)	n.s.	n.s.	n.s.	n.s.
Ipogepus dry matter (%)	n.s.	n.s.	n.s.	n.s.
Production of pods per plant (g)	n.s.	n.s.	n.s.	n.s.
Pod number (n)	n.s.	n.s.	n.s.	n.s.
Pod weight (g)	n.s.	*	n.s.	n.s.
Pod diameter (mm)	n.s.	n.s.	***	n.s.
1° order branch	**	n.s.	n.s.	n.s.

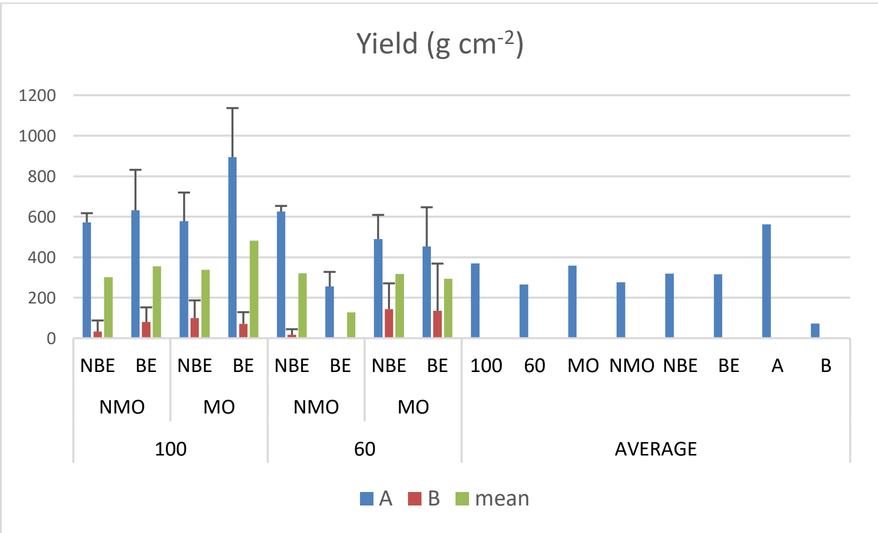
Results were presented at the ''*III International Organic Fruit Symposium and I International Organic Vegetable Symposium'':* Effects of microbial consortia and betaines on snapbean grown under water stress conditions.

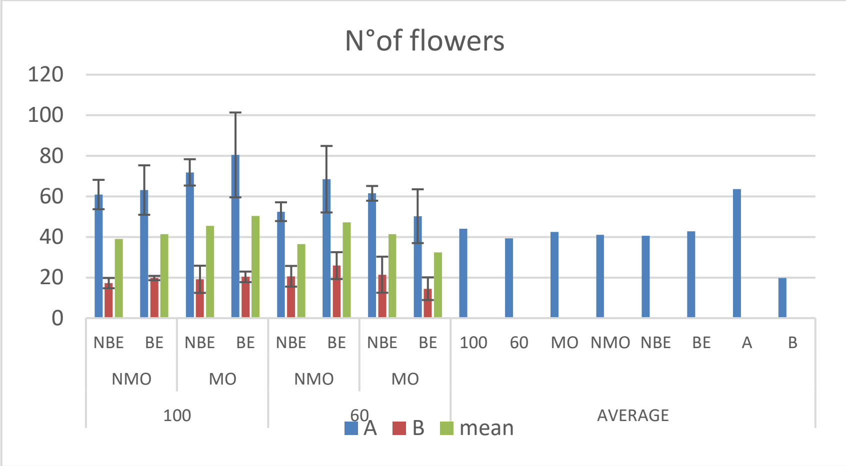
5.2.2. Effects of microbial consortia and betaines on greenhouse tomato grown under water stress conditions.

The trial was carried out during the growing period of winter 2021-22. The transplant took place on and seeds November 2021 in a cold greenhouse at the experimental farm of ITAKA srl located in Comiso (37°00'09.7''N, 14°.34'45.4''E). Seeds were sown on the sandy-loamy soil in rows with crop density 4 plants m^{-2}. The experimental design included 4 factors: i) water requirement based on crop evapotraspiration (ET_c) calculated by Pennman-Monteith formula; Application of microganisms (MO) using XP191BS by ITAKA srl; Application of betaines (BEs) using a commercial product; two cultivars (CV) of tomato (*Solanum lycopersicum*): 'Cherry ecologico'(A) and 'Flor de Baladre'(B).

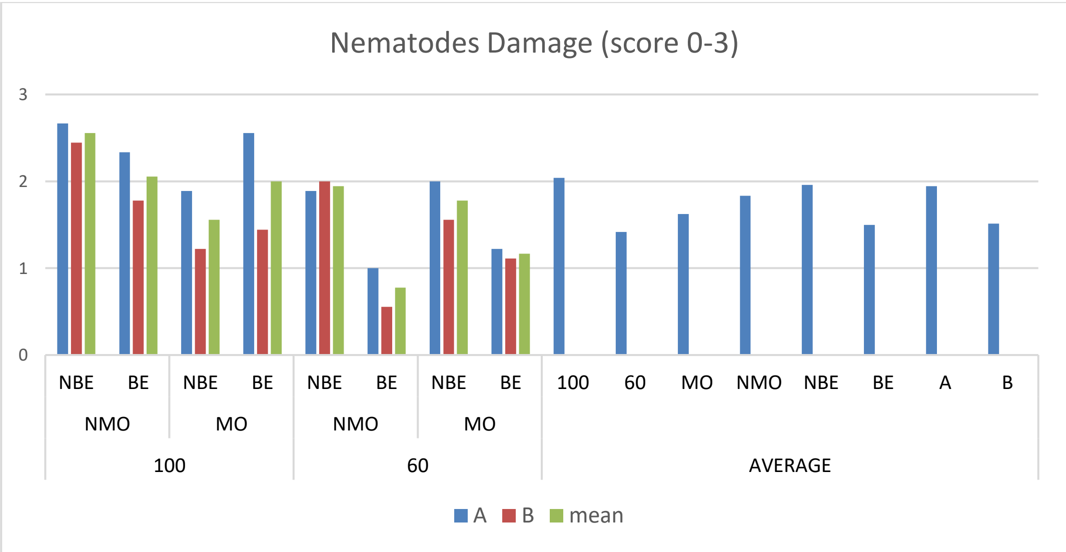
Results:

The test proceeded until the first 3 trusses were harvested and was then interrupted due to the high presence of *Tuta absoluta*. Cultivar A shown higher productivity both under optimal irrigation conditions and water stress. Both MO and BE influenced flowering and yield.





A slight and not significative reduction on nematodes damage was observed due MO and BE application.



Both trials confirm a trend linking the root system activity, plant development and yield. Betaines are confirmed to interact with resistance to water stress. The combination of factors, and most importantly microorganisms x betaines remain to be studied further.

5.3. Farmers' survey on the use of alternative fertilisers and microorganisms.

This survey looked at farmer's awareness on the use of non-chemical solutions as part of eco-sustainable production systems. Questions asked provide details on the work environment, the use and type of alternative fertilisers and microorganisms. We also asked for their feedback on results obtained and their satisfaction level.

46 persons responded to the survey from most areas in Europe. 37 completed the questionnaire.

5.3.1. General information:

Participants were coming from all regions in Europe with a strong presence from the Mediterranean and central areas (79%). This might be related to the geographic position of BRESOV partners who communicated about the survey. A majority of participants were directly involved with farming (69%) and for 81% of them in organic farming.

Participation/Region

Atlantic north	8%
Continental	11%
Mediterranean south	29%
Temperate climate (north med, Adriatic, central Europe)	50%
Other: West Africa	3%
Total	

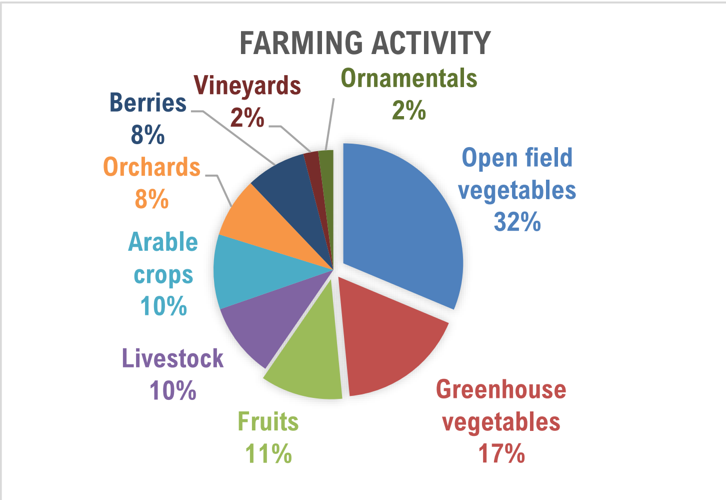
Profession:

Farm association	5%
Farm manager	18%
Farm technician	13%
Farmer	38%
Researcher	15%
Other	13%

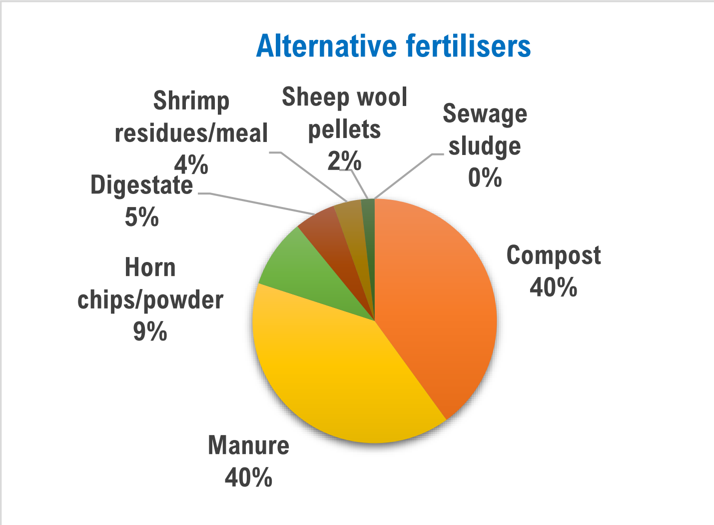
Farming systems

organic	63%	Organic = 81%
biodynamic/Demeter	5%	
in conversion to organic/Demeter	13%	
Integrated Pest Management	11%	Conventional = 19%
conventional	8%	

Crops reflected the area of the survey, with 60% of the respondents growing vegetables and fruits, of which 17% were in greenhouses. It should be mentioned that potato has been included in open field vegetables. Animal husbandry was present in 10% of the farms that responded to the survey. Other cultures mentioned by a few respondents were: olive trees, herbs, aromatic and medicinal plants, seed production and forest for feed.



5.3.2. Use of alternative fertilisers



Considering the types of alternative fertilisers available, 40% of people are using MANURE, consistent with the fact that 10% of the farms still have livestock, and that it is a requisite in Demeter guidelines.

COMPOSTS is used in 40% of cases, most probably purchased from local compost plants as it is often the case in organic farms.

61% of the participants confirmed they use alternative fertilisers as the only type of nutrition, which emphasizes the necessity to cover Nitrogen needs (51%). It also confirms the limited N sources available in organic farming and more generally the difficulty to balance N, P, K, Ca nutrition.

28% of people used alternative fertilisers as a complement to cover secondary needs such as micro-nutrients. A few other alternatives fertilizers were mentioned by the respondent: Insects dejections, beneficial soil microorganisms used as fertilizers, natural Sri Lankan eppawala rock phosphate, commercial products made from enzymatically hydrolysed animal skin components (BioEnne, Andis Bio-N), woodchips, sheep wool /new wool, as well as other commercially available organic fertilizers.

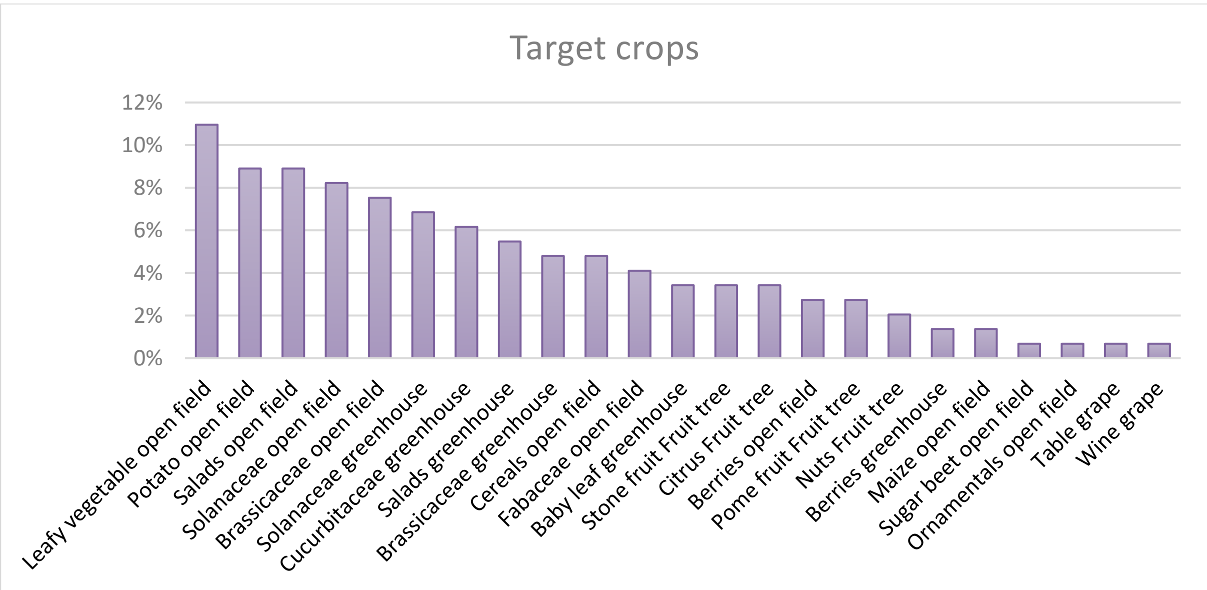
Proportion of the total fertilisation

25%	7%
50%	21%
75%	11%
100%	61%

Complement in priority

N	50%
Micro-elements	23%
P	12%
Don't know	8%
Ca	4%
K	4%

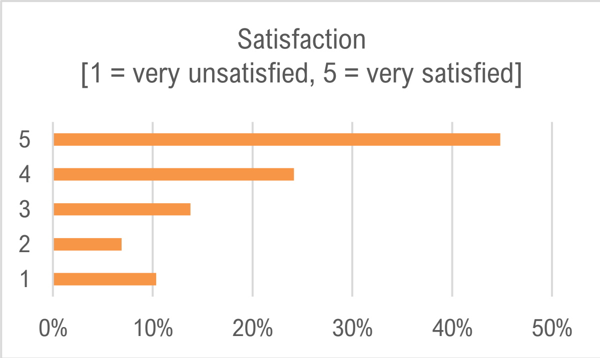
Target crops for alternative fertilisers are mostly open field crops (59%), the major ones being vegetables (45%), as opposed to greenhouse crops (28%), also in this case mostly vegetables. Vegetables (75%) are the overall main target for alternative fertilisers before orchards (12%) and arable crops (11%).



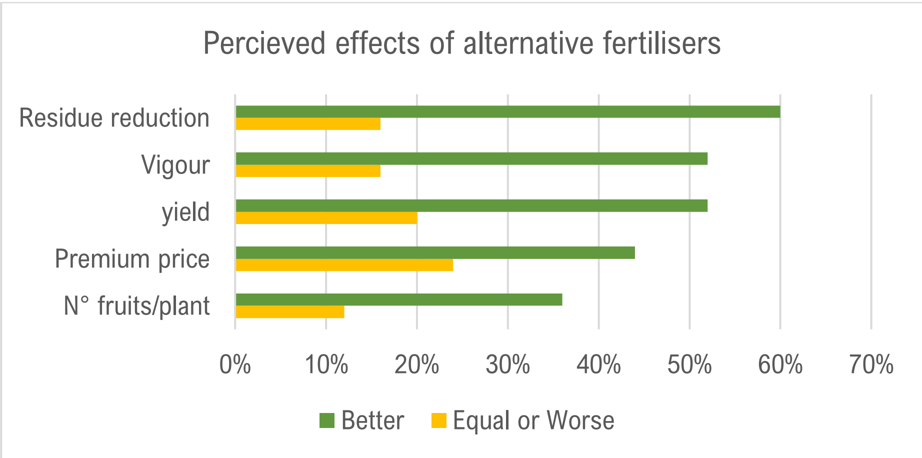
Others mentioned crops on which the alternative fertilizers are used are: carrots, spinach, grassland, beetroot, oil poppy, sweet potato, yacón and all open field cultures.

The satisfaction index on the use of alternative fertilisers is very positive; the addition of satisfied (4) + very satisfied (5) amounts to 69% of the people.

The two major areas of satisfaction are the residue reduction in food and premium price for quality, characterised by the highest "much better" observation and the lowest "don't know".

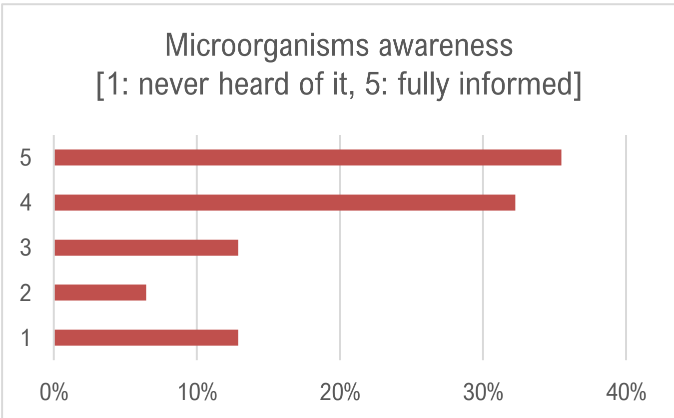


Overall, more than 50% of the people perceives the effect on residue, vigour and yield, which clearly have a value in front of the cost of using alternative fertilisers.



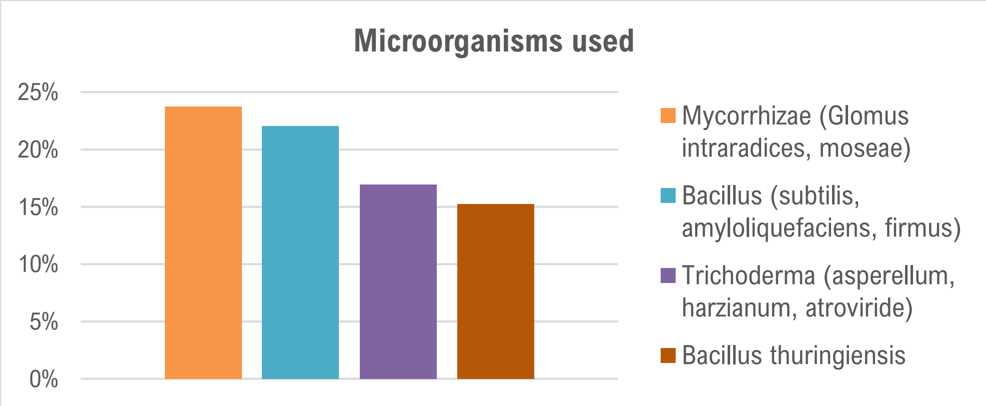
5.3.3. Use of microorganisms

More than 75% of people interviewed are aware of the use of microorganisms in agriculture. Similarly, to the results in the part of the survey on alternative fertilizers, the respondents of the survey are technically prepared and capable of running trials. Furthermore, 76% have tested microorganisms on their farms.



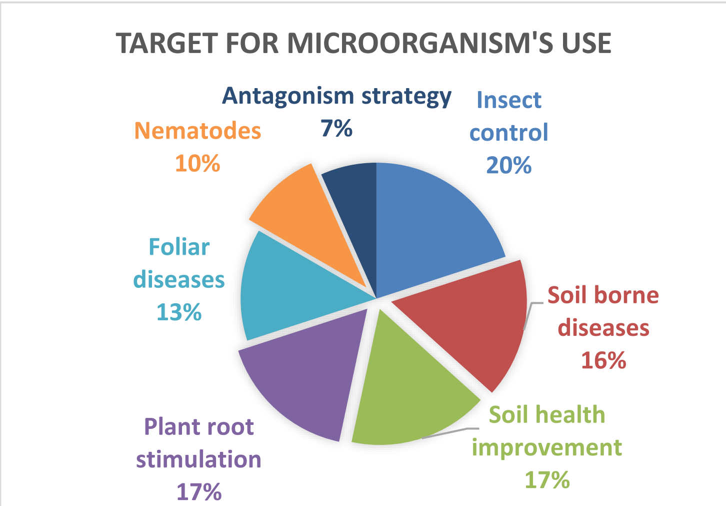
The understanding on the different types of microorganisms could be summarised as follows:

- Mycorrhizae: it is used as a general term; the type is not well identified.
- Bacillus species: subtilis and amyloliquefaciens are known
- Trichoderma species: asperellum, harzianum and atroviride are identified
- Bacillus thuringiensis is known.



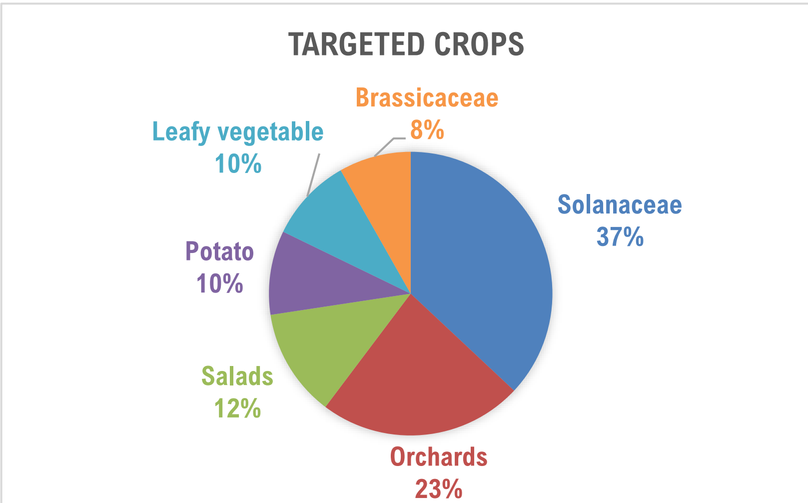
Other microorganism formulations mentioned by participants are: Bradyrhizobium (N fixing), Clonostachys sp (possibly *C. rosea* as biological control), yeasts, Phosphorus solubilizing microorganism, compost tea commercial mixes (EM- Schweiz), as well as other EM-Schweiz microorganisms' products.

An Irish farmer commented on the survey with the following feedback: "*use a singular micro-organism in your soil could potentially have dire consequences on the soil microbiome. We do not know the interrelationships between each species nor are we confident enough to confidently say that replacing one with another in singular fashion will have a beneficial impact in the long term*". This emphasizes indeed the need for research on effects of microorganisms in soils and on crops.



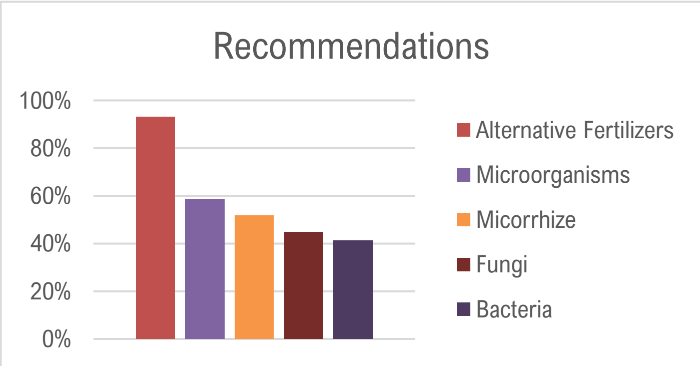
The responses to the survey confirm that people have a good knowledge of commercial products, their contents and main uses. In fact, the major use for microorganisms is insect control, using *Bacillus thuringiensis*. Then, soil applications, 60% of them using Mycorrhizae, Trichoderma and application against foliar diseases using Bacillus.

The crop repartition shows that farmers are aware of microorganisms and use them on all crops, in open field and greenhouses. Solanaceae in greenhouse and open field represents the major use (37%), namely tomato, green pepper and aubergine, followed by orchards, salads and potato.



The results from the comparison with conventional farming are unconclusive, as most respondents could not compare their results with those of farmers following another farming system. Moreover only 16% of the respondents were working in conventional farming /IPM. This underrepresentation in our survey reflects the network of BRESOV and its partner institutions and does not allow an unbiased comparison between the two systems. On the other hand, the few who answered indicated an increase in one or more of the criteria proposed: vigour, N° of fruits/plant, yield, premium price, residue reduction.

5.3.4. Recommendations



93% of the survey's respondents recommended the use of alternative fertilisers and 59% the use of microorganisms.

Despite this survey not covering the whole range of farming systems in terms of the number of answers and the representation of each type of farming, it provides an interesting insight on the use of alternative solutions. Farmers are interested and prepared to try and build experience, which bring them to use these solutions on most crops, contrarily to the general belief.

The perceived benefits are residue, vigour and yield, which are economically measurable.

The investment in dissemination activity, part of promoting eco-sustainable agriculture, should be completed by testimonials of successful experiences on technical viability and economical sustainability of applied responsible farming and results from monitored trials under different conditions to understand the underlying prerequisites for a successful application.

6. Conclusions

This task was designed to provide farmers with information and recommendations to support the transition to sustainable farming systems. It demonstrated, however, that dealing with soil health and its numerous interlinked variables, makes it difficult to give clear recommendations to the farming community on the use of alternative fertilizers and microorganism formulations, based on a set of trials over the short project period. Our trials have shown that dealing with living and variable products such as recycled fertilizers – mainly compost- and microorganisms' formulations can lead to very different results depending on product and context. As described in our published review, variability is introduced by the soil itself and by the environment and affect the microorganism survival or speed of development, and consequently the possible impact on plants.

Further investigations require the introduction of several parameters in the protocols:

- We have seen that **organic matter content** plays a role in the microbial activity, as well as the presence or deficiencies of nutrients.
- The **composition of microbiome** influences the development of introduced microorganisms.

This means that methodologies for microbial quantification and characterisation are extremely important and should be studied further. To cover the task would entail splitting the subject into more studies, in order to define better the conditions in which alternative fertilisers and microorganisms perform. For example: organic matter level and composition, definition/composition of a balanced microbiome for a given crop, influence of the introduction of new strains in the plant environment.

The farmers' survey added practical experience to the use of alternative solutions, confirming that it was possible to rely on alternative fertilizers and that there was a space for microorganism application in agriculture. It brought examples of success, showing that it is possible to reduce the dependence on chemicals. It also confirmed that alternative solutions require from farmers and technicians a good understanding of the mechanisms involved, and the necessity of advisory services to support the transition into more sustainable farming.

7. References

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