Development of biopesticide and biostimulant formulations for good agricultural practices in greenhouse cucumber cultivation

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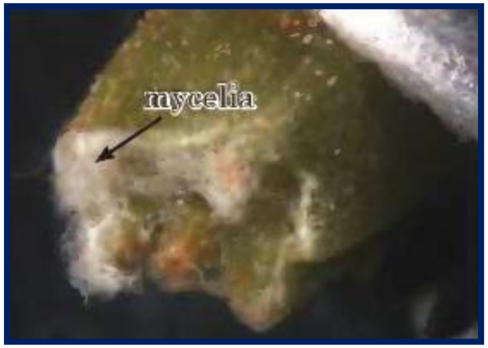
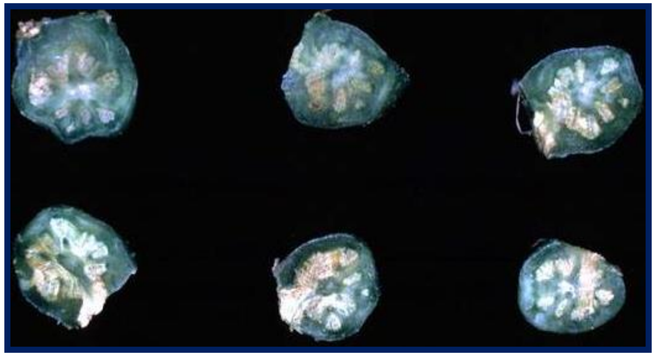
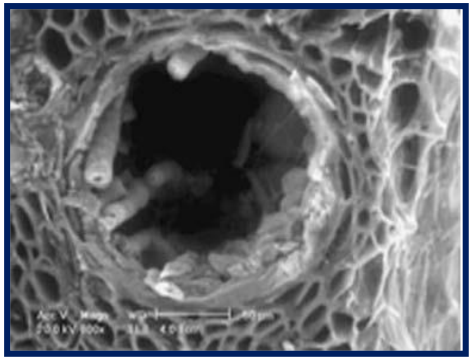
Greenhouse production is generally located on the coastal regions of Turkey. About 87% of the greenhouse production area in Turkey is concentrated in the Mediterranean region.

Fusarium oxysporum f. sp. cucumerinum

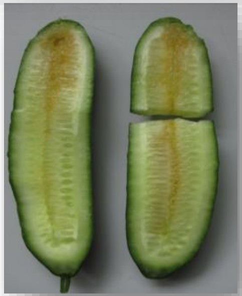
Fusarium oxysporum f. sp. cucumerinum, which is a soil-borne fungus, causes wilting and death of cucumber plants grown in greenhouse. Fungus colonizes the xylem vessels of cucumber plants as systemically and causes vascular discoloration







Angular Leaf Spot Of Cucumber (ALS) caused by ***Pseudomonas syringae* pv. *lachrymans (Psl)***

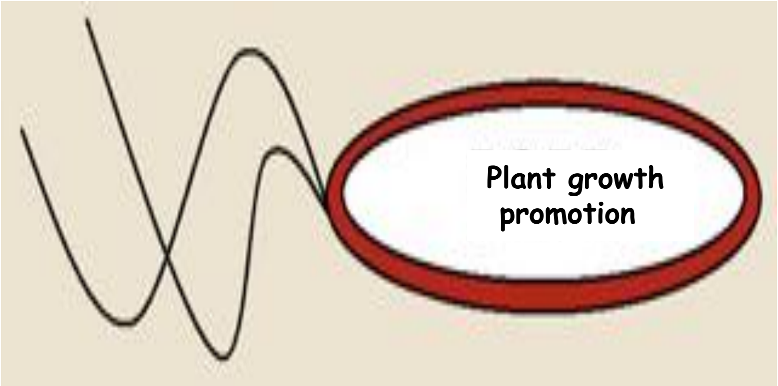


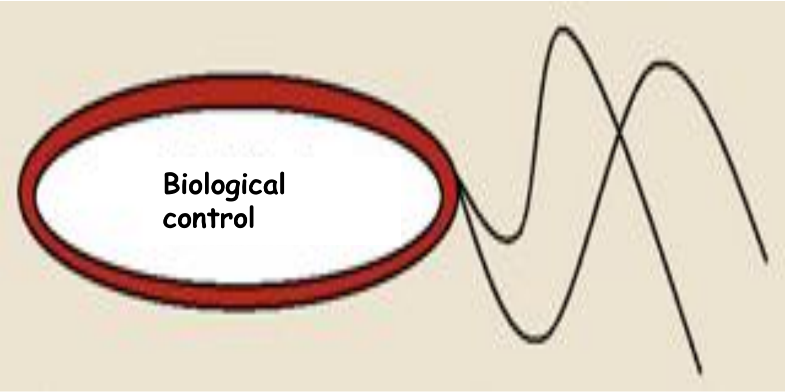
ALS is one of the most common bacterial disease on cucumber in all over world, results in significant yield losses in some seasons.

Systemic invasion of cucumber seedling stems, leaves, fruits and seeds by PSL is observed on pathogenic bacteria infected plants

Why biological control of FOC and PSL by endophytic bacteria

- It is difficult to control of FOC and PSL because this plant pathogens could cause systemic invasion
- There is no resistant cucumber cultivars to FOC and PSL
- **Biological control** has been considered as an potential method for controlling the plant diseases
- The use of **endophytic bacteria (EB)** to control plant-pathogenic bacteria is receiving increasing attention as a sustainable alternative to synthetic pesticides.





•increasing the availability of nutrients for the plant,
•production of the phytohormones,
•degrading the toxic compounds
•suppressing of ethylene production by ACC deaminase activity

Inhibiting fungal activity and antagonizing bacterial pathogens

Backround of the project

1. Isolation and identification of EB
2. *In vitro* and *in vivo* efficacy tests of EB for biocontrol and plant growth promotion

SAMPLING OF HEALTHY CUCUMBER PLANTS FOR THE EB ISOLATON

FROM GREENHOUSES

FROM FIELDS

Isolation of endophytic bacteria

Step 1	Step 2	Step 3	Step 4	Step 5
5% sodium hypochlorite for 10 min.	70% ethanol for 5 min.	Rinsed with Sterile Distilled Water	Rinsed with sterile distilled Water	Rinsed with sterile distilled Water Check the contamination

Isolation of endophytic bacteria

Two ways for EB isolation

Trituration

The tissue was macerated into 100 ml phosphate-buffered and plated onto **TSA** plates

Imprinting of tissue

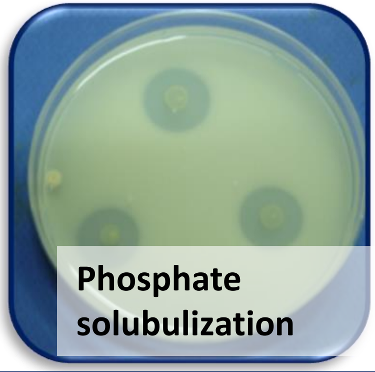
Stems and roots, 2–3 cm long, were cut vertically or longitudinally with a scalpel. The pieces were transferred and pressed onto TSA, left for 1 h, and then removed

IN VITRO TESTS

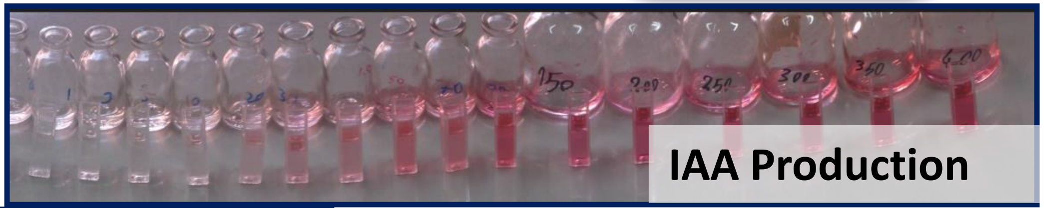
Isolated EB strains were screened *in vitro* for their plant growth promoting and biocontrol activities:

- **PLANT GROWTH PROMOTING TRAITS**
  - Production of IAA (Brick et al., 1991)
  - Production of HCN (Lork,1948)
  - Siderophore Production (Schwyn and Neilands, 1987)
  - Phosphate solubulization (Gaur,1990)
  - Effect on vigority Index of cucumber seeds (Nejad and Johnson,2000)
  - ACC deaminase activity
- **BIOCONTROL ACTIVITIES**
  - Antifungal activity against FOC and PSL

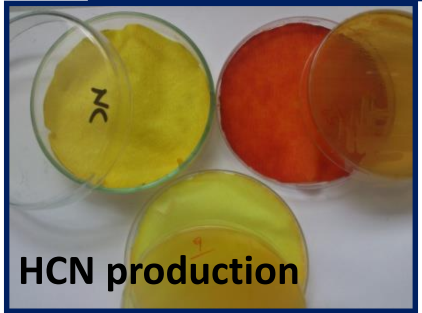
Siderophore Production



Phosphate solubulization

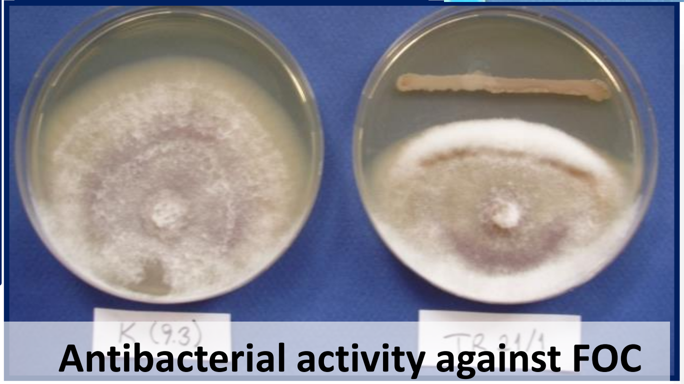


IAA Production



HCN production

Vigority index

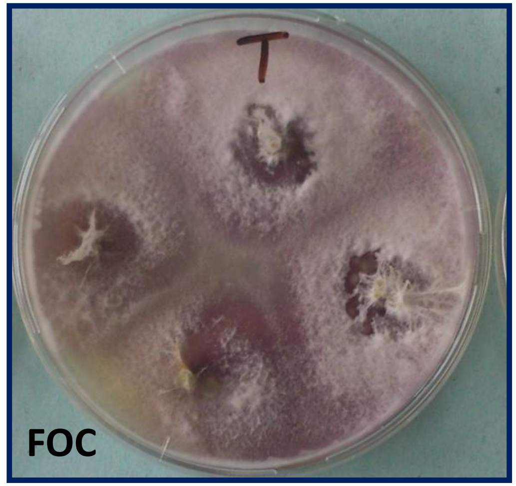


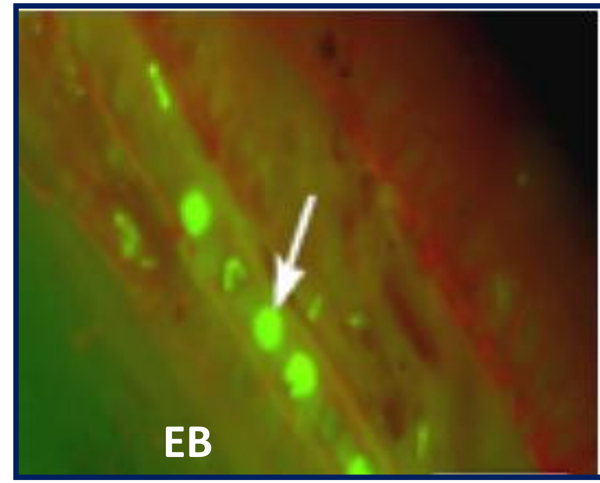
Antibacterial activity against FOC

Antibacterial activity against PSL

AIM OF THIS RESEARCH

The final goal in biocontrol studies is to obtain bioformulations with high stability and effectiveness. The main aim of this study was to provide the commercial use of endophytic bacteria, which were successful for biological control of Fusarium wilt, angular leaf spot and growth promotion of cucumber cultivation

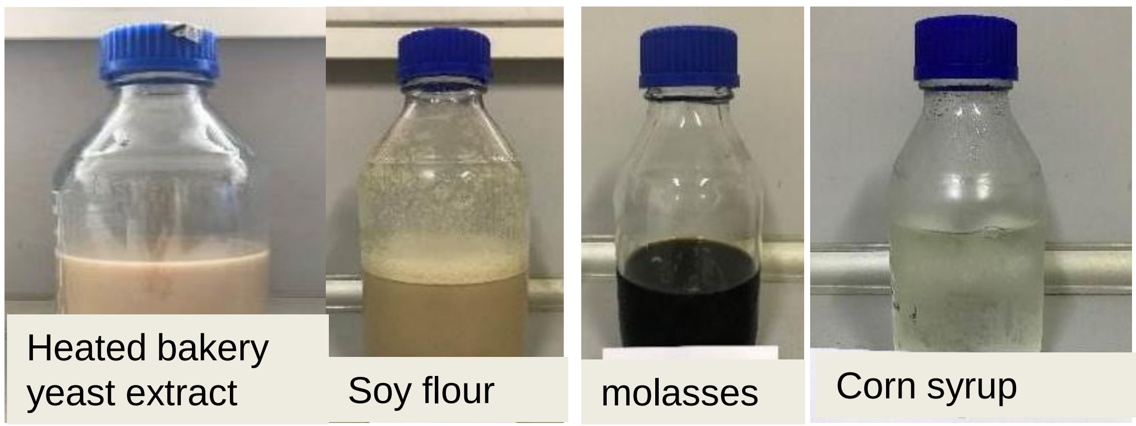


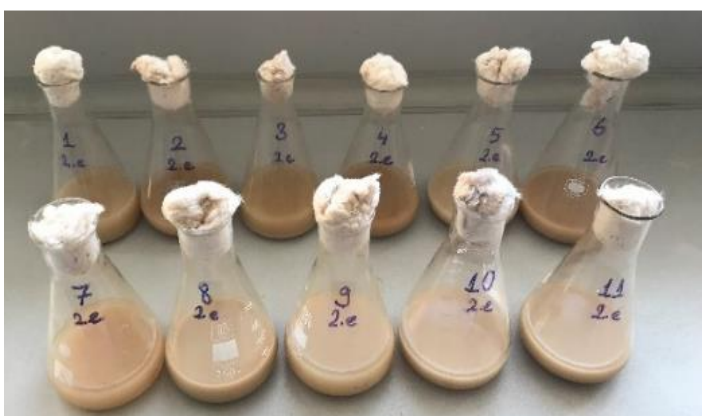


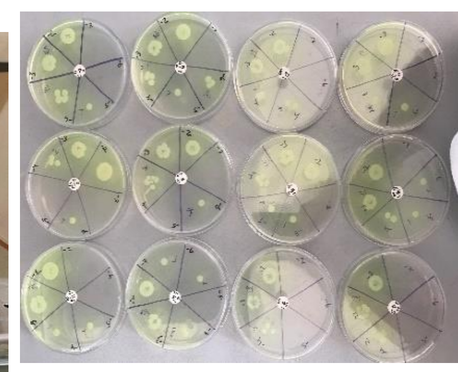
BIOPROCESS & BIOFORMULATION OF EB

Scale up production of beneficial bacteria

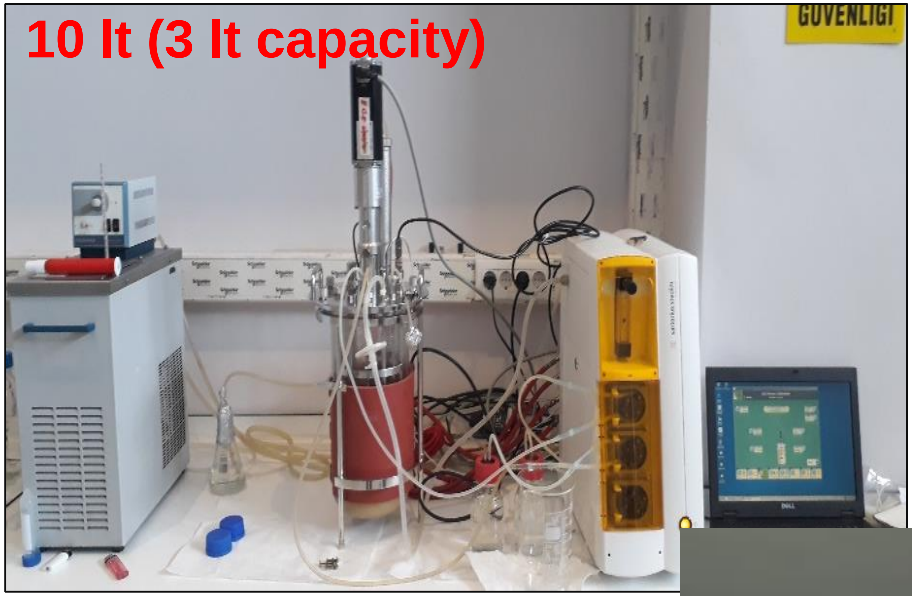
The EB strains were grown on cheaper substrates such as molasses, skim milk, heated bakery yeast extract, corn syrup, soy flour as liquid medium for 24 h as a shake culture incubated in a rotary shaker at 120 rpm at room temperature (24 ± 1°C). Liquid NB medium was evaluated as a control growth medium. Population density of each EB strain in each tested was monitored by plate dilution method. The most suitable growth medium was selected for scale-up production of each EB in 10 and 100 L bioreactor.





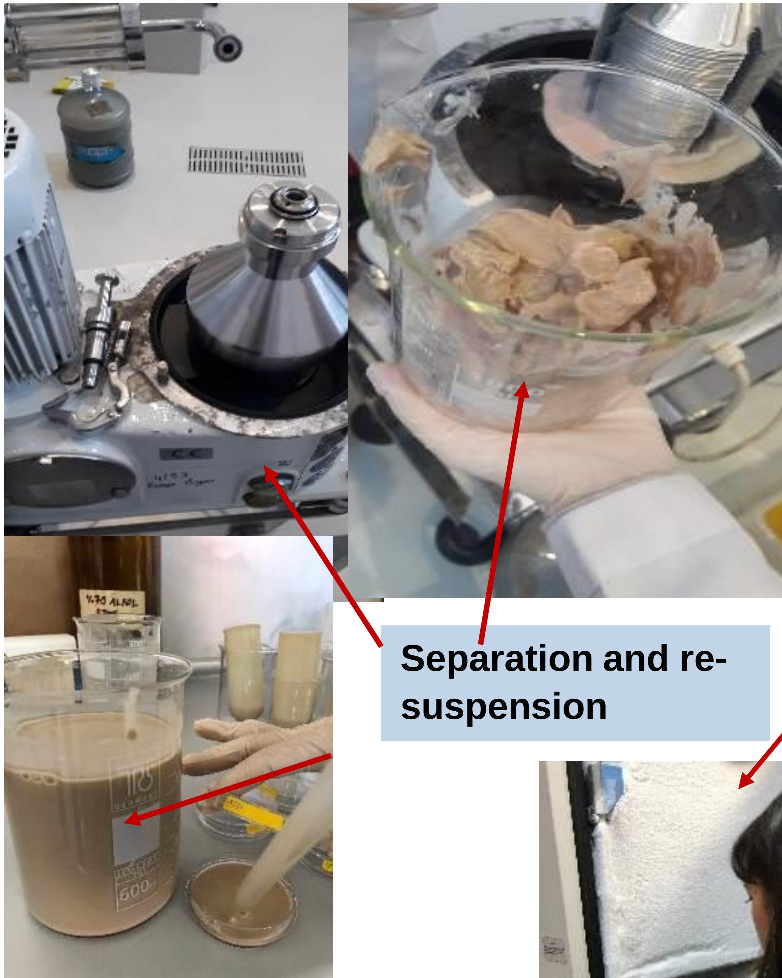


Scale – up process of EB in bioreactors



Scale-up process: 10 (3 liter capacity) and 100 liter reactors (30 liter capacity) used in scale-up production phase

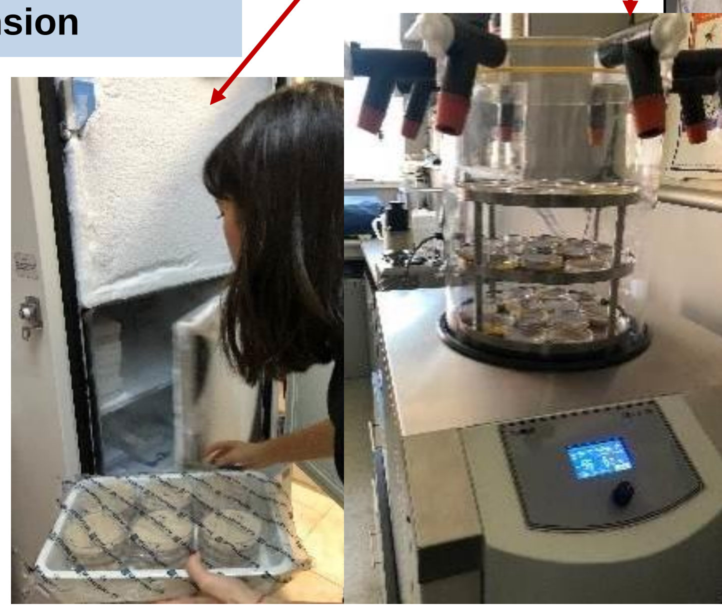
FORMULATION AND MONITORING OF SHELF-LIFE OF BENEFICIAL BACTERIA

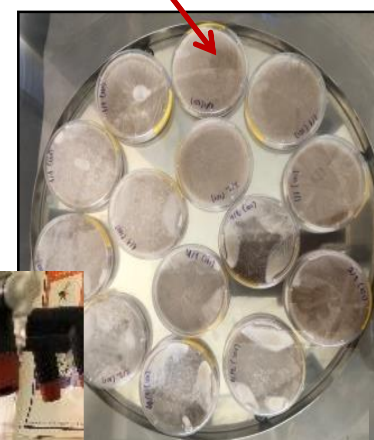


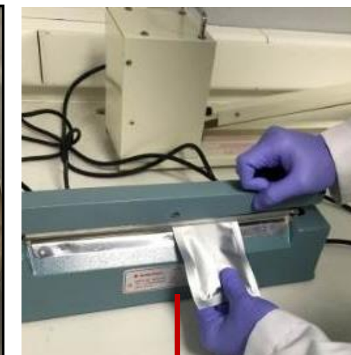
- Bacterial biomass was then separated, and pellets were suspended in skim-milk (10% w/v).
- The bacterial pellet was dried in a freeze-drying machine for 24 h to obtain wettable powder (WP) formulations of tested EBs.

freeze-drying

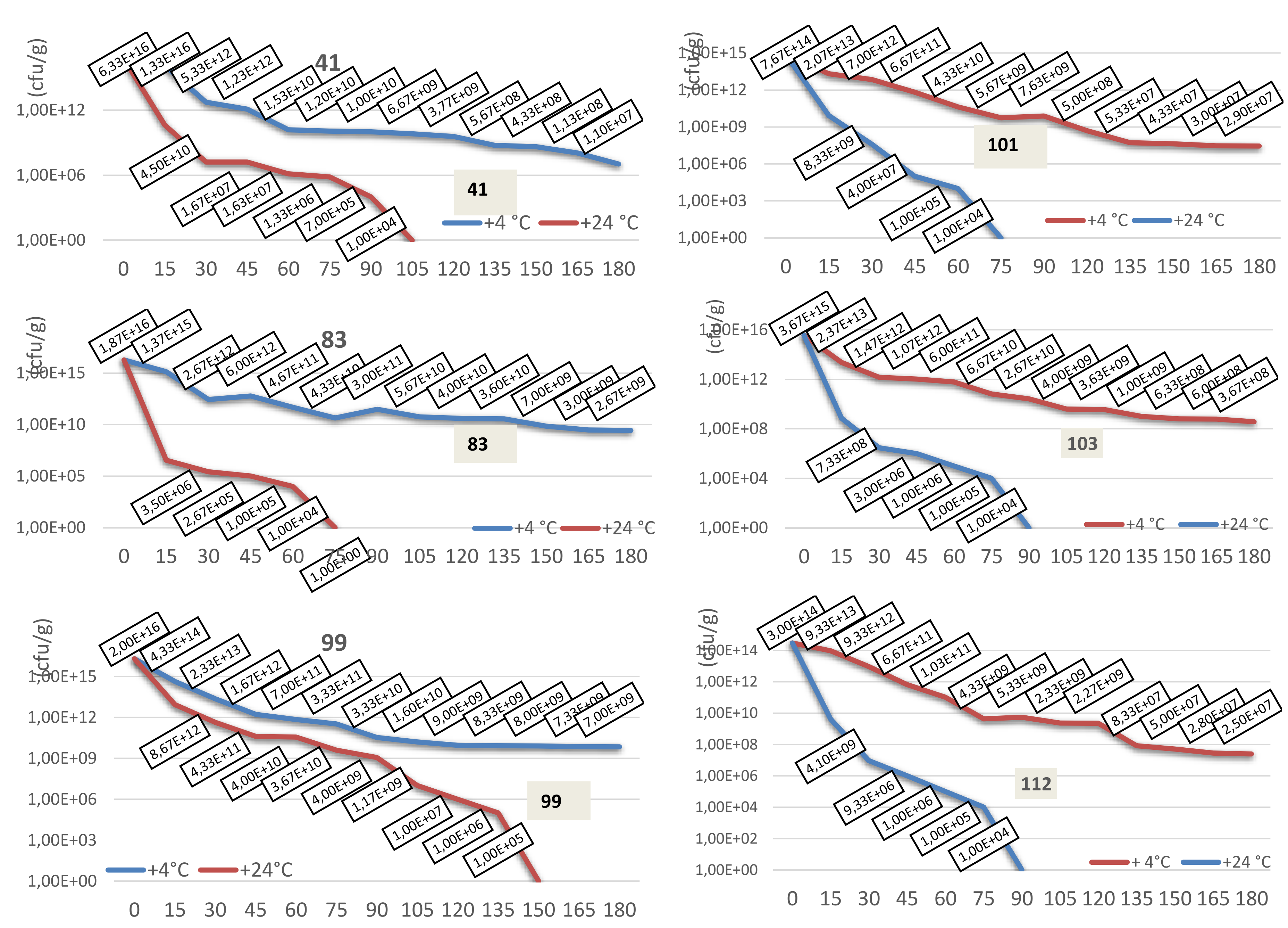
packaging







Monitoring the shelf life of bioformulations for 180 days



TESTING OF EB FORMUALATIONS FOR BIOCONTROL AND GROWTH PROMOTION of Cucumbers UNDER *IN VIVO* CONDITIONS

1. POT TESTS IN CLIMATE ROOM CONDITIONS
2. GREENHOUSE APPLICATIONS

IN VIVO TESTS:
IN GROWTH CHAMBER ROOM

- BIOCONTROL OF FOC and PSL by EB
- GROWTH PROMOTION OF CUCUMBER PLANTS by EB

Effect of EB formulations on Fusarial wilt, angular leaf spot and growth promotion of cucumber by pot tests




Seed coating by EB formulations (10^8 CFU ml^{-1}) and sowing into peat




Preparation of FOC spore suspension (10^5 spore ml^{-1}) and transplanting of plants to FOC infested soil

- The EB formulations took place twice before sowing as a seed coating and after transplanting as substrate drenching
- EB treated cucumber plants were transplanted to FOC inoculated soil
- Wilt development on each plant was rated 21 days after transplanting to the FOC infested pots using the 0-4 scale.
- The effects of EB formulations on growth promotion of cucumber plants were also evaluated by weighing the fresh and dry weight of shoots and roots of EB treated plants without any disease pressure

Soil drenching by EB formulations (20 ml per plant, 10^8 CFU ml^{-1}) after trnsplanting

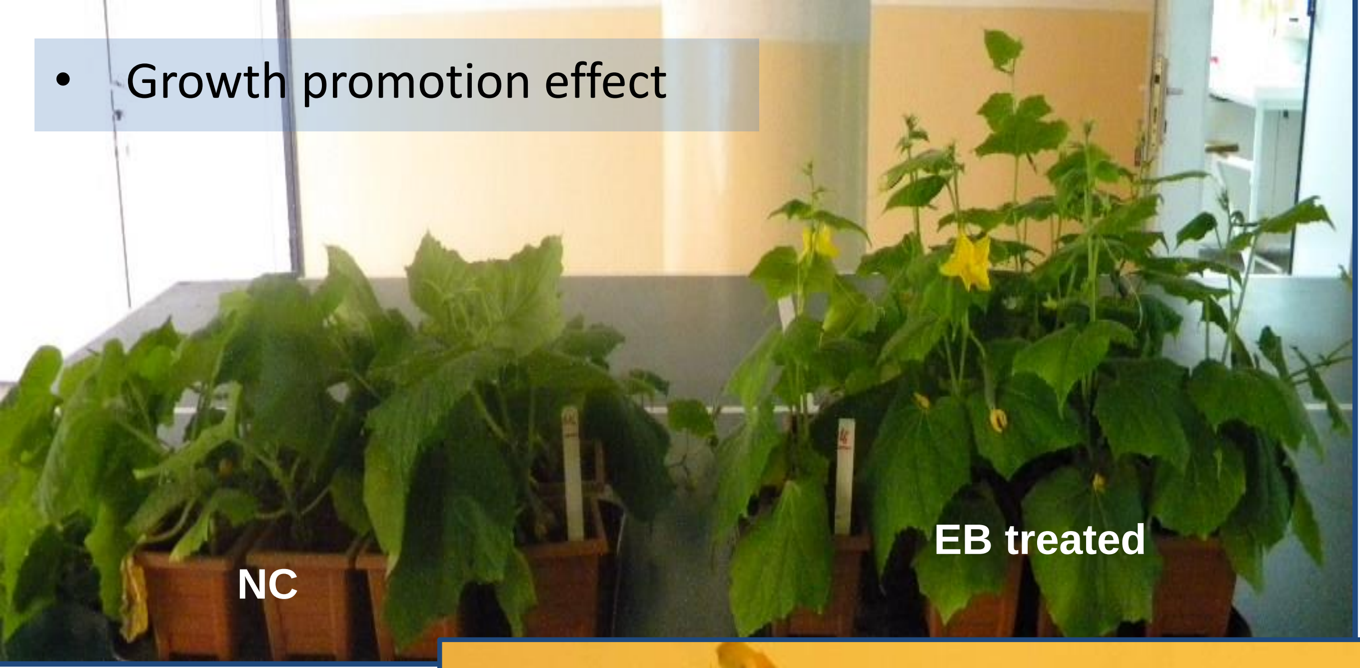
Effects of the EB formulations on *F. oxysporum* f. sp. *cucumerinum* and growth promotion of cucumbers by pot test

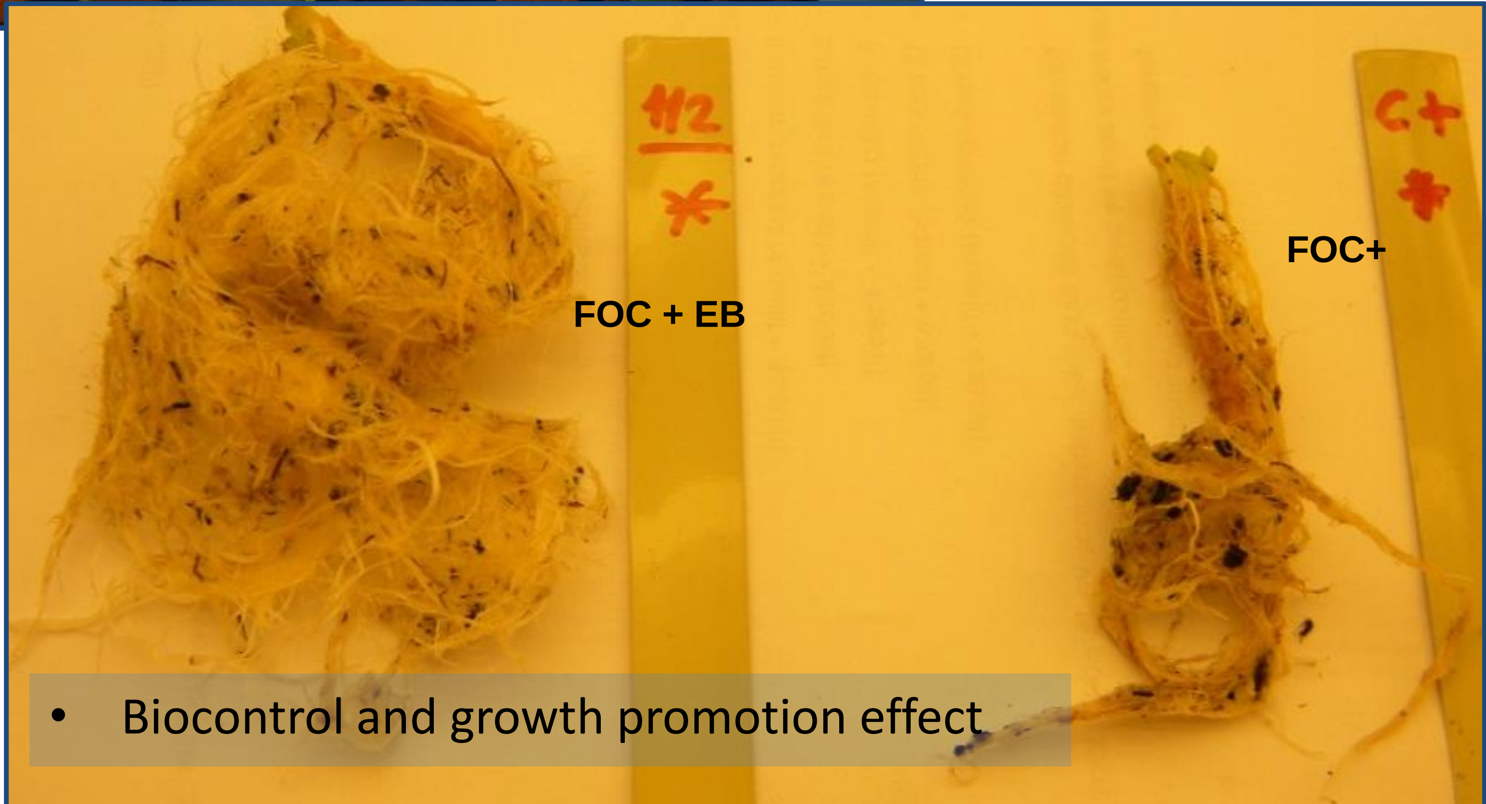
EB No	Mean wilt severity (%)*	Efficacy (%)***	Total biomass (g per plant)*			
			Total fresh weight		Total dry weight	
			Root	Shoot	root	shoot
41	65 cd**	21.69	12.90 a**	13.50 bc**	0.59 a**	1.27 ab**
83	**15 ab**	**81.93**	**13.32 a**	**15.19 ab**	**0.68 a**	**1.34 ab**
99	46 bc	44.58	15.48 a	13.81 abc	0.62 a	1.30 ab
101	30 abc	63.86	10.54 ab	13.78 abc	0.55 a	1.17 b
103	85 d	00.00	4.72 b	10.15 c	0.28 b	1.11 b
112	45 bc	45.79	5.25 b	10.15 c	0.25 b	0.87 c
Positive Control	83 d	---	-----	----	---	--
Negative control	0 a	---	9.30 ab	17.53 a	0.49 ab	1.56 a

EB formulations exhibited the Fusarium wilt reduction between 22 to 82 % compared to only FOC inoculated plants according to growth chamber test results. The most promising EB treatment was *P. agglomerans* strain 83. Morover, EB strain 83, without being any disease pressure, increased the total fresh shoot and dried shoot and root biomass compared to non-treated cucumber plants.

In planta tests

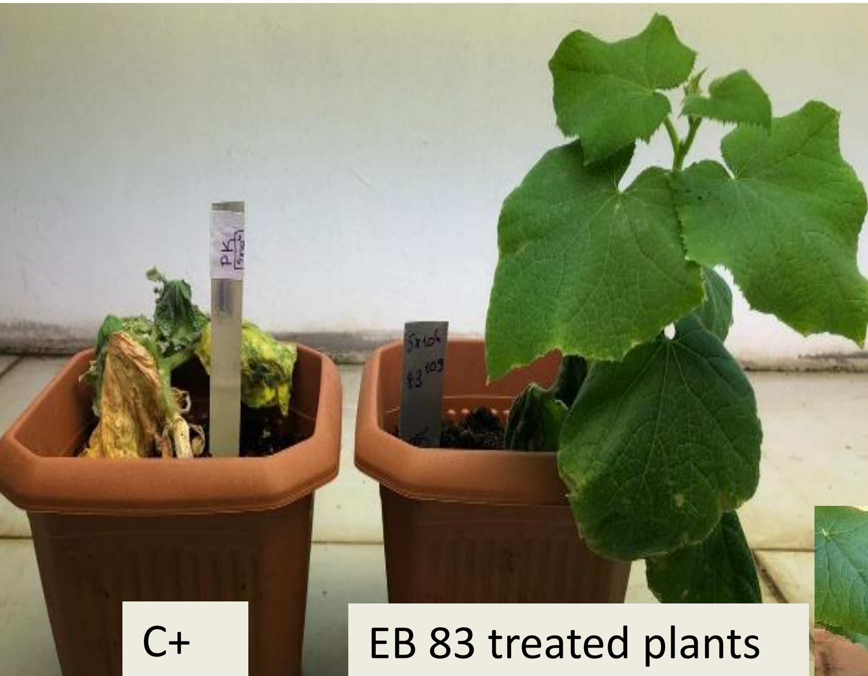
- Growth promotion effect

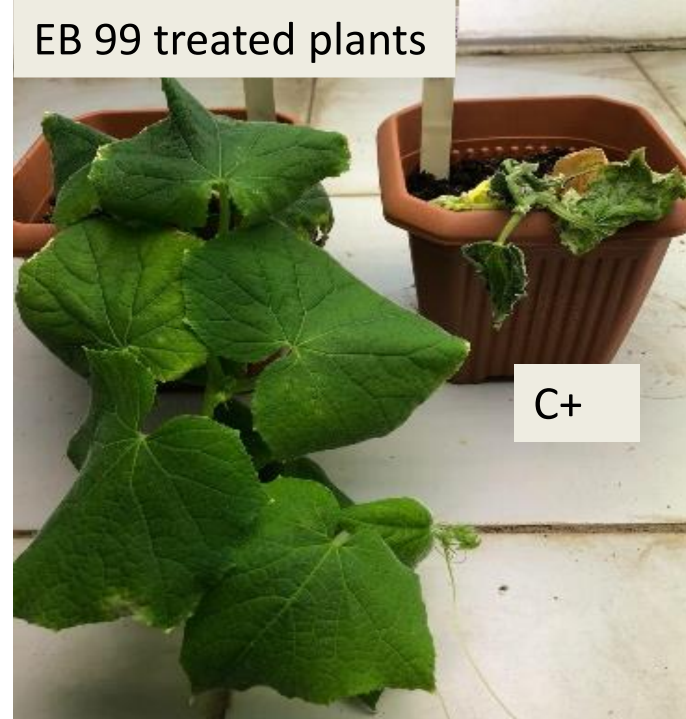




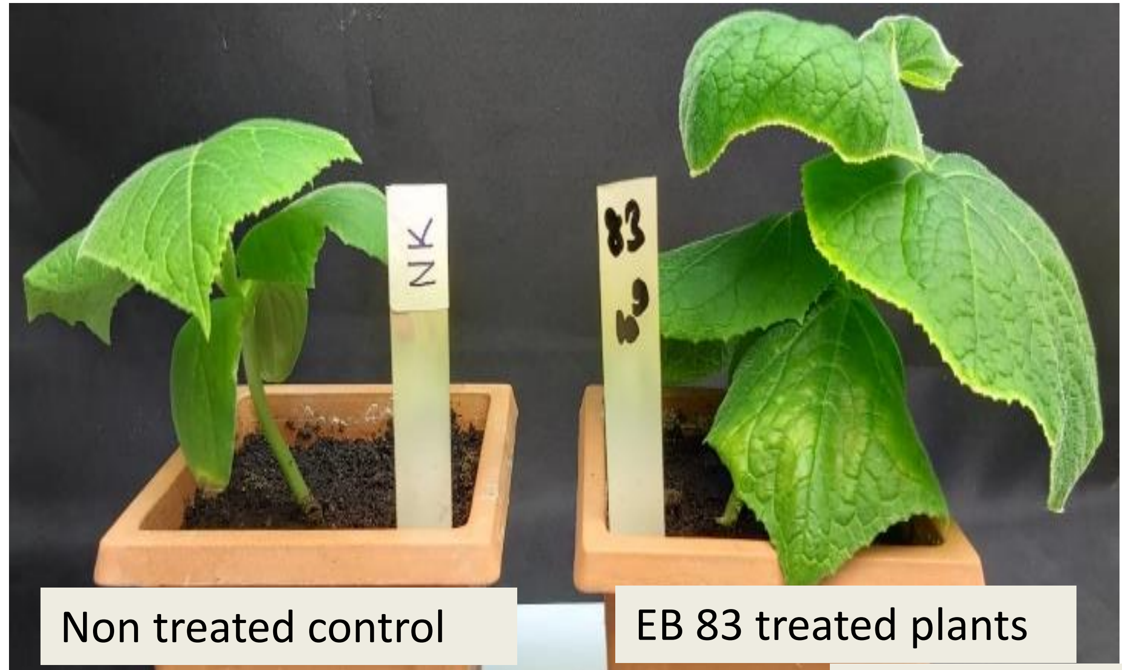
- Biocontrol and growth promotion effect

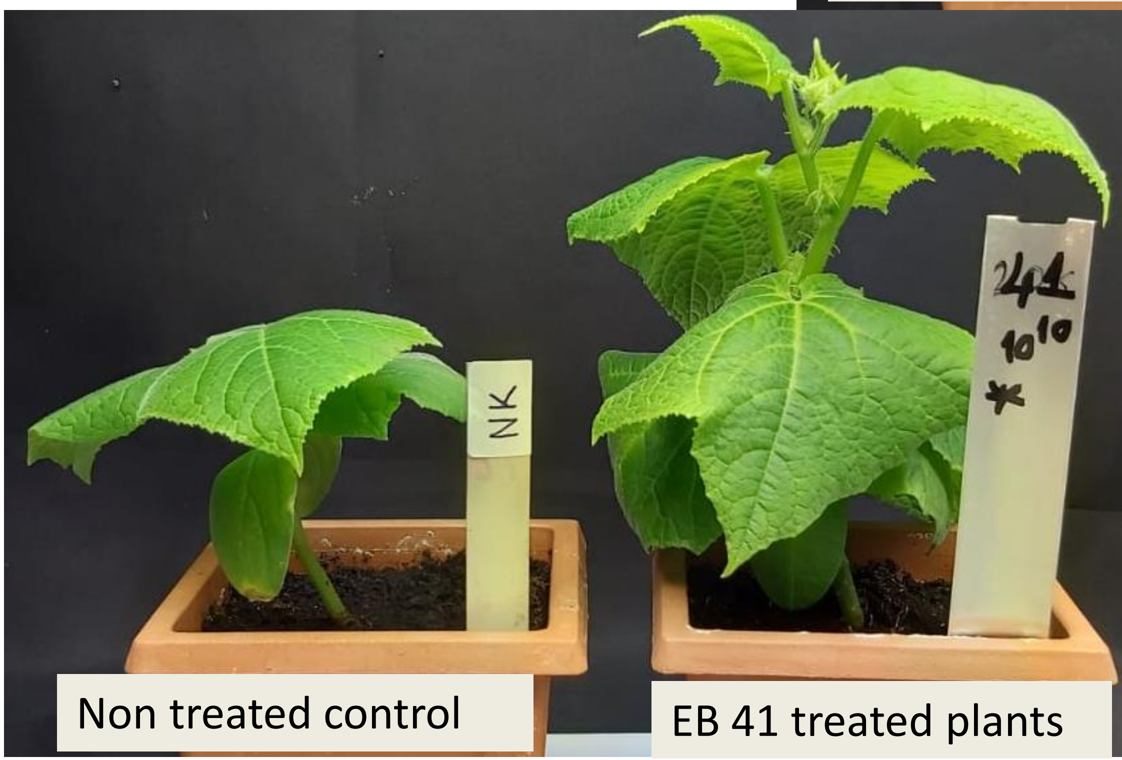
Effects of EB formulations on FOC development by pot tests





Effects of EB formulations on PLANT GROWTH PROMOTION by POT TESTS





GREENHOUSE TREATMENT TO TEST THE BIOCONTROL of FOC OF SELECTED EB FORMULATIONS

- Cucumber plants with treated EB as seed bacterization and soil drenching before transplanting to peat inoculated with FOC spore suspension,
- Four weeks cucumber plant were transferred to soilles medium pots for greenhouse experiment
- EB treatments were repeated by soil drenching after transferring the perlite medium of seedlings

Soil drenching treatment

- We monitored the cucumber plants in terms of disease development of FOC 2 months after transplanting to soilless system

Effect of bacterial formulations on FOC in cucumber cultivation in the soilless agriculture greenhouse of the Plant Protection Department

Treatments*	Effect of bacterial formulations on FOC in cucumber plants	
	Mean disease severity (%)**	Efficacy (%)****
83	**68.75 b*** **	**23.25**
112	93.75 c	--
83+112	87.50 c	2.32
PC	89.58 c	--
NC	0,00 a	--

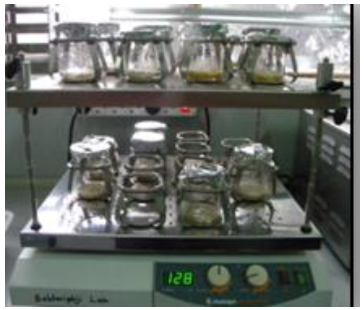
**Values are the average of 6 replicates with 2 plants each and the disease severity values according to the 0-4 scale, 8 weeks after the seedlings were transplanted into the soilless farming system in the greenhouse.
***According to Duncan's multiple comparison test, the difference between the means shown with the same letters in the same column is insignificant according to $P < 0.05$
****Values show the difference (%) of the mean disease severity (%) values from the Positive Control according to ABBOTT.

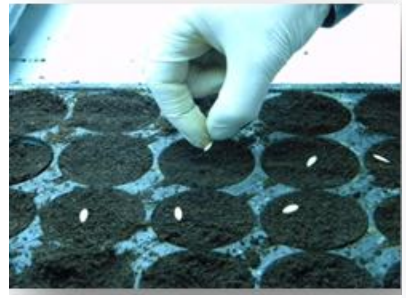
Pathogen (FOC) alone treatment

EB TREATMENTS

EB treatments applied as seed coating and soil drenching inhibited the Fusarial wilt development at the rate of **49 to 52%** compared to only FOC inoculated cucumber plants for two months growing period in soilless growing system.

Application of EB for biological control of *Pseudomonas syringae* pv. *lachrymans*





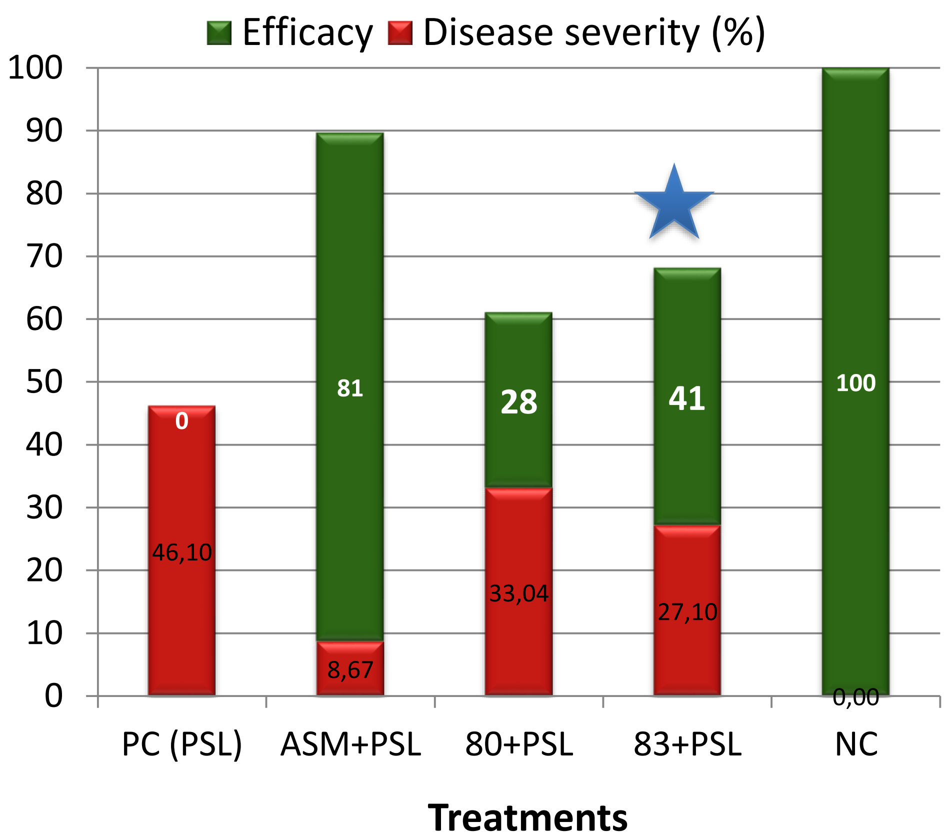
EB were applied twice: seed coating and soil drenching.

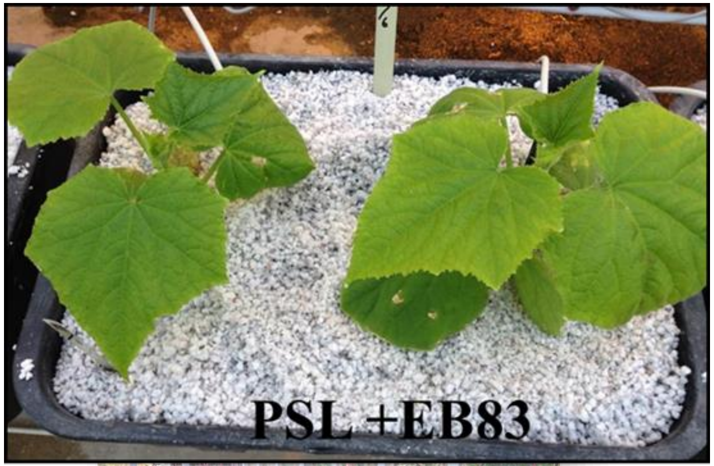
Plants were sprayed with PSL, and kept in a climate chamber for 4 days

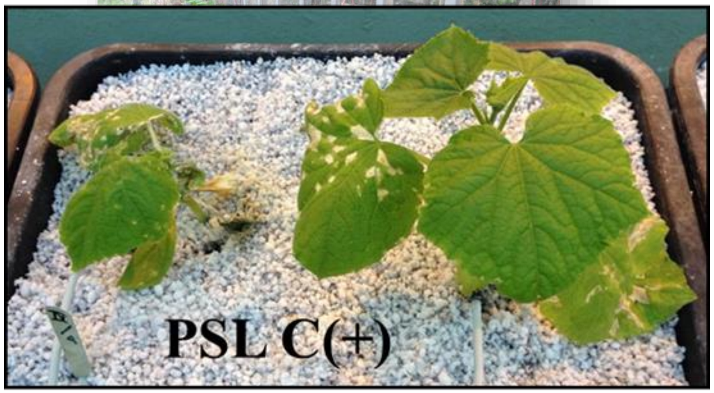
The seedlings were transplanted at soilless growing system

- The disease severity was evaluated by 0-4 scale 21 days after PSL inoculation in soilless growing system

Disease severity of PSL in soilless growing system





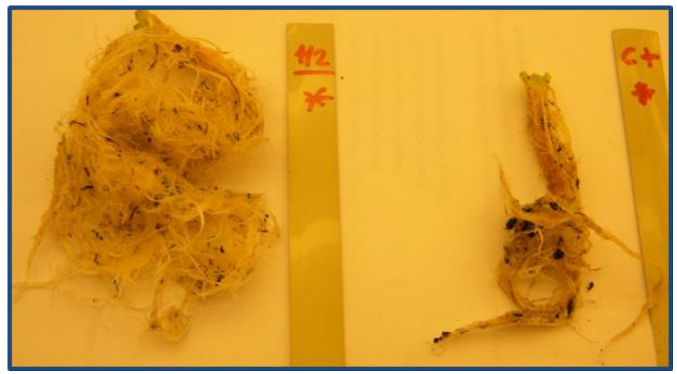


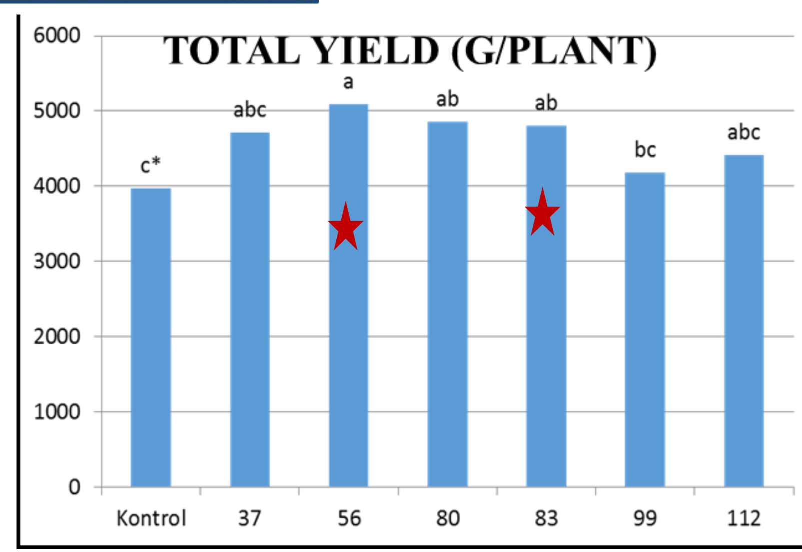
ASM (Bion R) was used as chemical control.

Disease severity of ALS caused by PSL was limited by **EB80 (28%)** and **EB83 (41%)** in soilless growing system.

Effects of EB on growth promotion and yield increasing

Some of *EB* strains, without being any disease pressure, increased the total marketable fruit yield at the rate of 5 to 28%, compared to non treated cucumber plants in soilless cultivation





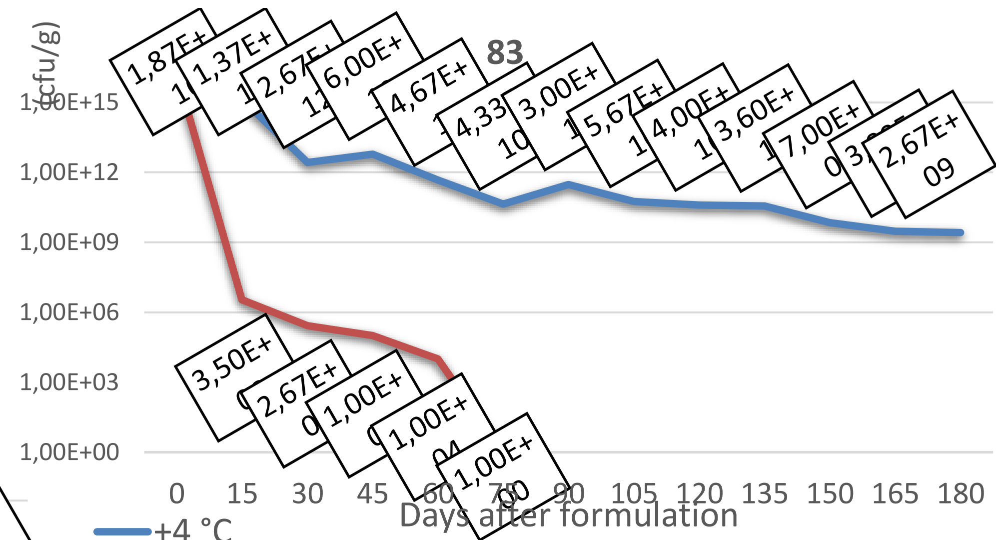
In conclusion

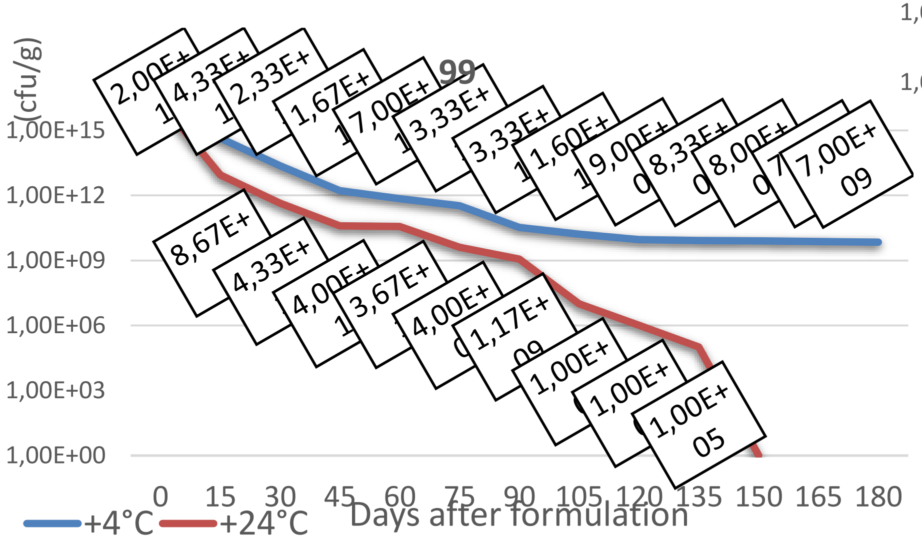
SOME STRENGTHS AND OPPORTUNITIES (POSITIVES)

- In this study, it was proved that tested formulations of EB strains were found very promising for plant growth promotion and biocontrol parameters.
- The results of our previous study indicated that EB strains 83 **(*Pantoea agglomerans*)** and 99 **(*Bacillus thuringiensis)*** produced indole 3-acetic acid (IAA), siderophore, aminocyclopropane-1-carboxylate (ACC) deaminase, and solubilise phosphate for plant growth
- So, the application of wettable powder formulations of EB strains at the concentration of 10^{7}-^{8} cfu per ml^{-1} as a seed coating and soil drenching was recorded as effective and practical techniques in terms of biological control and growth promotion of cucumber plants.

SOME LIMITATIONS AND CHALLENGES (NEGATIVES)

- The insufficient viability and shelf life of bioformulations is a weakness compared to chemical formulations.
- Failure to extend viability and shelf life up to 1 year is a deficiency.
- It is necessary to test some additives to the formulation for a more stable and longer shelf life
- It should be investigated cheaper drying and formulation techniques other than freze drying technique





TAHNKS FOR YOUR ATTANTION

SOME LIMITATIONS AND CHALLENGES (NEGATIVES)

- Local companies do not invest enough in bioformulation production and do not attach enough importance to R&D.
- Large-scale of production problems for microbial products couldn’t be solved yet in Turkey.
- Commercialization and legislation problems cannot be overcome in Turkey.
- Obtaining a biopesticide license in Turkey is a bureaucratically long and expensive process.
- So, it is easier to obtain a license for bioformulations as a biofertilizer or biostimulant in Turkey
- The Ministry of Agriculture is insufficient to encourage and support farmers in the use of biological products.