# BACTERICIDE ACTION OF CERTAIN BIOLOGICAL PRODUCTS

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*Under laboratory conditions the bactericide action of certain biological products proceeded from three stems cultures of Bacilus subtilis were studied. The toxicity of antagonistic stem cultures against pathogenic bacteria Agrobacterium tumefaciens, Pseudomonassyringae pv,tomato of agriculture plants were tested in vitro. The action of the three stems using the culture medium inclusion method showed that the inhibitory action percentage and calculation limit concentration and corelation coefficient. The results of our studies it is recommended the use of the following bio-preparation: bactericide product ICC F84 + ICCF 284 – isolated of couchgrass and dried cereals; ICC F84 + ICCF 382 – isolated of couchgrass and barley; ICC F84 +ICCF 285 - isolated of grass and medicinal. Limit concentration 50% situated a value 2%. Biological products has been experiments in control crown galls by Momordica cultivated in greenhouse, through application seed treatments.*

***Key words:*** *pathogenicus bacteria, biological products, biocontrol*

The biological means utilized on cultivated plants diseases prevention and control become one of the main concern in finding the methods and tools to diminish the damages caused by the pathological organisms, as well as environmental pollution. The biological means practical application lead to the intensified study about microbial antagonism. Finding the antagonists dispersion it might be touched the pathogenesis limitation of theirs hosts, and the diminishing the production loss. [1, 2]. Other studies in the field refer to some microorganism inhibitory action such as fungi (*Penicillium, Aspergillus, Chaetomium*) and bacteria (*Bacillus sp., Echerichia coli, Erwinia amylovora*) on pathogenic bacteria classified as *Agrobacterium, Pseudomonas, Erwinia* [3,4,5] A good efficacy of tumorogenesis prevention has been achieved by non-virulent *Agrobacterium* stem, which in the field prevented the cultivated plants infection, by the exclusion of virulent stem from the penetration place HLB-2 stem, biovar 1, isolated by Xiaoyang, China, that inhibited cancer in high percentage [6, 7, 8].

## MATERIAL AND METHOD

The bio-preparations proceeded by the *Bacillus subtilis* delivered by National Research -Development Institute Chimic/Farma -Bucharest have been tested as:

ICC F84 + ICCF 284– isolated of couch grass and dried cereals;
ICC F84 + ICCF 382 – isolated of couch grass and barley;
ICC F84 + ICCF 285 - isolated of couch grass and medicinal plants;
B4 - isolated of foliage forest leafs.

The phyto-pathogenic test bacteria:

*Pseudomonas seringe pv. tomato*, *Xanthomonas campestris pv.vesicatoria, Erwinia carotovora* and *Agrobacterium tumefaciens*, cultivated on the peptone + glucose + agar culture medium.

The *in vitro* method of inclusion bio-preparation in culture medium the bactericide action of pathogenic bacteria has been tested. Aiming setting the best bactericide action concentration, four concentrations added in culture medium for each biologic product have been tested (concentration of 4%, 3%, 2%). The results were validated by statistical means correlation coefficient (CC), establishing the limit concentratin (CL). With the aim to fight against this bacteriosis a study regarding the pathogen control in soury cucumber seeds was initiated. A biologic product ICC F84 + ICCF 285 was selected based on results of the *in vitro* investigations. More specifically, the seeds were maintained for 24 hours immersed into 4% ICC F84 + ICCF 285 solution before planting. Subsequently, the seeds were sown in pots and after plantlets were transplanted into the open fields.

## RESULTS AND DISCUSSIONS

As the result of our, in table 1 is shown the following test bio-products: ICCF84 + ICCF284 – isolated of quick grass and dried cereals; ICC F84 + ICCF 382 – isolated of quick grass and barley; ICC F84 + ICCF 285 - isolated of quick grass and medicinal plants; B4, have been inhibited the development of bacterial colonies of *Pseudomonas seringe pv. tomato, Xanthomonas campestris pv.vesicatoria, Erwinia carotovora* and *Agrobacterium tumefaciens*, up to 4% concentration. Under this concentration for example at 2% the colonies have developed, the medium surface was occupied between 23 and 73% (in table is noted as inhibitory percentage) .

The product based on ICCF84 + ICCF284 stem showed a strong inhibitory action, the bacteria colonies developed only at 2% concentration, showing an inhibitory process of 75% for *Xanthomonas campestris pv.vesicatoria,* 47% for *Agrobacterium tumefaciens,* 43% for *Pseudomonas seringe pv. tomato* and 38% for *Erwinia carotovora.*

The results of our studies recommended the use of the following bio-preparation: ICCF84 + ICCF284 – isolated of quick grass and dried cereals; ICCF84 + ICCF382– isolated of quick grass and barley; ICCF84 + ICCF285 - isolated of quick grass and medicinal. Limit concentration 50% situated a value 2 (*tab.1)*.

Under greenhouse conditions the soury cucumber (*Momordica*) cultivated as medicinal plant had exhibited plants drying during vegetation period in pre-floral phase, symptoms which continued also after fruit formation. Sick plants showed

quite big tumors on roots (till 10 mm diameter, 10 cm lenght and 3-4 cm width). Also, seeds from these plants when cultivated *in vitro* on peptone, beef extract and agar medium formed *Agrobacterium tumefaciens* typical colonies.

It was noticed that plantlets, coming out from the treated seeds showed an increased vigour compared with the untreated ones. Besides, their growth was aslo good and when planted into greenhouse did not show any bacterial symptoms. Compared with the testimony (untreated seeds) where the *Agrobacterium tumefaciens* attack reached 28%, the plants obtained from ICCF84 + ICCF285 treated seeds were totaly healthy, without any disease symptoms. Moreover, into the soil of the greenhouses cultivated with medicinal plants the presence of *Agrobacterium tumefaciens* was inevitable due to the polivor character of the pathogen which attacks all dicotiledons.

## CONCLUSIONS

1. The following bio-preparations had been *in vitro* selected: ICCF84 + ICCF284 –isolated on couchgrass and dry cereals; ICCF84 + ICCF382– isolated on couchgrass and common barley; ICCF84 + ICCF285–isolated on couchgrass and medicinal plants; B4 which at 4% concentration inhibited the development of bacterial colonies of pathogens *Pseudomonas syringae pv. tomato, Xanthomonas campestris vesicatoria, Erwinia carotovora* and *Agrobacterium tumefaciens,* cultivated on artificial media (peptone+glucose+agar) in which the biologic product had been included. At concentration lower than 3% bio-product bacterial colonies had been formed depeding by the bio-product used. In presence of ICCF84 + ICCF284 (included into the atrificial media) a very high inhibition was registered 75% for *Xanthomonas campestris pv.vesicatoria*, 47% for *Agrobacterium tumefaciens,* 43% for *Pseudomonas tomato* and 38% for *Erwinia carotovor* species.

2. From our studies, we reccomend the use of the following bio-products: BSP+BSU – isolated on couchgrass and dry cereals; ICCF84 + ICCF382 – isolated on couchgrass and common barley; ICCF84 + ICCF285–isolated on couchgrass and medicinal plants; B4, at 4%concentration, in order to protect the agricultural crops against the attack of the bacterial pathogens: *Pseudomonas syringae pv. tomato, Xanthomonas campestris pv.vesicatoria, Erwinia carotovora* and *Agrobacterium tumefaciens.*

3. Under greenhouse conditions the soury cucumber (*Momordica*) manifested during the vegeation period, a dry symptoms of plants in pre-floral phase, symptoms which continued also after fruit formation. The attacked plants exhibited quite big tumors on roots (10 mm diameter, till 10 cm lenght and 3-4 cm width). Seeds from affected plants placed on artificial media (peptone+beef extract+agar) formed colonies specific to *Agrobacterium tumefaciens.*

4. The pathogenic action of *Agrobacterium tumefaciens* bacteria at soury cucumber (*Momordica),* cultivated as medicinal plant under greenhouse conditions, was controlled by applying a seed treatment with the biologic product ICCF84 +

ICCF285 at 4% concentration (water diluted).The treatment consisted in seeds immersion into the above mentioned solution 24 hours before planting. After the treatment, the seeds were planted into soil cubes in order to obtain seedlings.

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Table 1

**Biological action of bio-preparation on the pathogenic bacteria colonies development**

| Biological products | Conc. (%) | *Pseudomonas syringae* pv. *tomato* | | | *Xanthomonas vesicatoria* | | | *Agrobacterium tumefaciens* | | | *Erwinia carotovora* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Inhibitory action % | CL*- 50% | CC** | Inhibitory action % | CL*- 50% | CC** | Inhibitory action % | CL*- 50% | CC** | % de Inhibitie. | CL*-50% | CC** |
| ICCF84 + ICCF382 | 4.0 | 100.00 | 2.29 | 0.895 | 99.95 | 2.01 | 0.976 | 100.00 | 1.97 | 0,927 | 98,97 | 2,07 | 0,968 |
| | 3.0 | 74.04 | | | 83.14 | | | 87.54 | | | 87,79 | | |
| | 2.0 | 25.74 | | | 51.36 | | | 68.53 | | | 53,91 | | |
| B 4 | 4.0 | 97.85 | 2.73 | 0.938 | 99.52 | 2.51 | 0.897 | 98.57 | 2.98 | 0,945 | 95,91 | 2,71 | 0,876 |
| | 3.0 | 75.93 | | | 68.93 | | | 52.11 | | | 79,59 | | |
| | 2.0 | 32.59 | | | 24.53 | | | 17.99 | | | 29,45 | | |
| ICCF84 + ICCF285 | 4.0 | 95.57 | 2.03 | 0.950 | 92.78 | 2.99 | 0.919 | 100.00 | 2.69 | 0,879 | 99,94 | 2,12 | 0,995 |
| | 3.0 | 79.12 | | | 51.96 | | | 77.19 | | | 82,95 | | |
| | 2.0 | 45.91 | | | 31.06 | | | 38.95 | | | 43,33 | | |
| ICCF84 + ICCF284 | 4.0 | 100.00 | 2.37 | 0.972 | 100.00 | 1.78 | 0.999 | 100.00 | 2.70 | 0,935 | 99,78 | 2,67 | 0,984 |
| | 3.0 | 96.41 | | | 89.57 | | | 81.11 | | | 61,25 | | |
| | 2.0 | 46.89 | | | 75.00 | | | 48.23 | | | 38,96 | | |
| Check | - | 0.00 | - | - | 0.00 | - | - | 0.00 | - | - | 0,98 | - | - |

*CL - Limit concentration **CC - Corelation coefficient