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BIOCONTROL OF FUSARIUM WILT OF CUCUMBER (*CUCUMIS SATIVUS* L.) BY *BACILLUS* SP.

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ABSTRACT

The aim of this study is to test the effectiveness of three bacterial strains M12, M23 and M21 of *Bacillus sp* genus isolated from roots of Mint (*Mentha rotundifolia* L.) on the stimulation of agronomic parameters (height, fresh weight and dry weight) of Cucumber, as well as their ability to reduce the appearance of Fusarium wilt of Cucumber (*Cucumis sativus* L.) caused by *Fusarium oxysporum*. Concerning the growth stimulation, the *Bacillus sp* strain M21 presents a highly significant effect on all agronomic parameters and save the highest values of the growth promotion efficiency (GPE) as compared with the other bacterial strains M23 and M12. It has a GPE (%) of 71.18; 62.79; 80.79, 81.51; 65.44 and 84.37 respectively for air length, root length, air fresh weight, fresh root weight, air dry weight and root weight. Regarding the disease severity, *Bacillus* sp. M23 showed a highly significant reduction in the disease incidence with a percentage of 13.11 %, this corresponds to a significant biocontrol efficacy of 67.22 % against *Fusarium oxysporum*. In contrast, *Bacillus* sp. M12 presented the lowest value of the biocontrol efficacy against Fusarium wilt which only reached 33.35%. Consequently, the strains of *Bacillus* sp. M21, M23 and M12 genus have a stimulatory effect on agronomic parameters, and a protective effect against Fusarium wilt of Cucumber. These two effects place it among the bacteria PGPR (Plant Growth Promoting Rhizobacteria).

KEYWORDS: biological control, Fusarium wilt, Cucumber, *Bacillus* sp., *Fusarium oxysporum*, PGPR.

INTRODUCTION

Fusarium wilt of Cucumber is a serious fungal disease caused by *Fusarium oxysporum*. Typical symptoms of wilt disease in Cucumber plants include necrotic lesions, vascular wilt, and roots wilt, which induce serious yield losses.[1]

The control of fungal diseases is mainly based on chemical control. However, the use of pesticides is not recommended for the diseases transmitted by the soil due to their high cost and low efficiency. In addition, pesticides can be toxic for humans, animals and crops, and thus lead to the development of tolerant pathogenic fungicide strains.[2, 3]

The biological control of diseases transmitted by the soil is an important alternative for chemical control. It provides an effective way to control the disease with less harmful effects on the environment.[4, 5, 6]

This alternative may be based on the application of microorganism populations such as endophytic bacteria. These are advantageous for plants.[7] They stimulate growth[8, 9]; reduce the disease severity by inducing plant defense mechanisms.[10, 11, 12]

Among bacterial endophytes, the bacteria of the *Bacillus* sp. genus expressed significant antagonistic activities for *Fusarium oxysporum* also in vitro and vivo.[13]

The objective of this study is to test in vivo the effect of three endophytic bacterial strains of *Bacillus* sp. genus on Fusarium wilt and their ability to promote the growth of Cucumber plants.

MATERIALS AND METHODS

Preparation of the inoculums

The endophytic bacterial strains used were isolated in the laboratory of Biology, Health and Environment (Applied Microbiology Team), from the roots of the Mint plant (*Mentha rotundifolia* L.). These are strains of Bacillus sp (M23, M21 and M12).[14]

The bacterial inoculum of each strain adjusted to the concentration of 10^9UFC/ml with24 hours of planting on nutrient broth. The incubation is performed at28°C.[15]

The phytopathogenic *Fusarium oxysporum* mycelium was incubated for 15 days in the PDA medium. The spores are obtained by flooding the culture with 1 ml sterile distilled water and

then scraping the medium surface with the tip of a sterile Pasteur pipette. The suspension gotten put in 20 ml of distilled sterile water. The spore concentration adjusted to the concentration of 10^9 spores/ml.[16] The incubation in the dark at 28°C.

Fusarium oxysporum was provided by The Laboratory Botany and Plant Protection (LBPP) of the Faculty of Sciences: Ibn Tofail University -Kénitra.[17]

Disinfection of Cucumber seeds

Disinfection of Cucumber seeds (*Cucumis sativus* L.) was performed according to the modified method of Gotz and al. (2006).[18] The seeds are immersed in ethanol (70%) for one min followed by sodium hypochlorite (12%) for 15 min, then washed several times with sterile distilled water. The seeds were pre-germinated *in vitro* for three days.

Antagonistic effect of *Bacillus sp* strains on cucumber *in vivo*

The pre-germinated seeds were soaked in different bacterial suspensions (10^9UFC/ml) of *Bacillus sp* (M23, M21 and M12) for 2 hours.

Three seedlings of each treatment were transplanted into pots containing Mâamora soil either sterile or infected with *Fusarium oxysporum*.

The cultures were maintained in a greenhouse at a temperature day/night of about 25/18°C and 75% relative humidity for 30 days. Plants were regularly irrigated with tap water.
Two controls were used: A= the seedlings were treated with sterile distilled water.
B= the seedlings were treated with *Fusarium oxysporum*.

Determination of agronomic parameters

After Forty days of cultivation, the agronomic parameters of the harvested plants are measured.

RATING RESULTS

In order to determine the effect of antagonists isolates on the growth plant compared to the control, the growth promotion efficiency (GPE) was calculated by the following formula.[19]

$$GPE(\%) = \frac{GT-GC}{GC}$$

With GT: growth parameter of plants treated with the strain,
GC: growth control parameter.

The disease incidence and effectiveness of biological control were calculated according to the following formulas.[20]

$$\text{Disease incidence (\%) (D.I)} = \frac{\text{(The disease Index x Number of diseased plant having this index)}}{\text{(Number of studied plants x the highest Index of the disease)}} \times 100$$

$$\text{Effectiveness of biological control (\%) (B.F)} = \frac{\text{(Index of disease control- disease incidence of antagonist treated group)}}{\text{(Index of disease control)}} \times 100$$

The estimated disease incidence basing on the scale of Mu (2000)[21] ranging from 0 to 4:

0: the leaves show no symptoms.

1: <20 % of leaves with symptoms.

2: 20-50 % of the leaves with symptoms.

3: 50-80 % of the leaves with symptoms.

4: >80 % of leaves with symptoms.

Statistical analysis

The data were statistically processed by analysis of variance (ANOVA) and means were compared using the least significant difference (LSD) method; $P \leq 0.05$.

RESULTS AND DISCUSSION

Evaluation of agronomic parameters of Cucumber (Cucumis sativus L.)

The stimulation ability Tests of the plants growth of Cucumber with *Bacillus sp* (M23, M12 and M2) showed in Table (1) and Figure (1).

Table 1: *In vivo* Efficacy of *Bacillus* sp. (M21, M23 and M12) on the growth parameters of Cucumber (*Cucumis sativus* L.)

	Air parameters						Root parameters					
Treatment	**Length (cm)**	**GPE (%)**	**Fresh Weight (g)**	**GPE (%)**	**Dry Weight (g)**	**GPE (%)**	**Length (cm)**	**GPE (%)**	**Fresh Weight (g)**	**GPE (%)**	**Dry Weight (g)**	**GPE (%)**
M21	60*	71.18	54.33*	80.79	5.41*	65.44	49*	62.79	45.67*	81.51	4.72*	84.37
M23	44.5*	26.96	40.91*	36.13	4	22.32	45*	49.50	28.91*	14.90	3.2	25
M12	42*	19.82	36.78*	22.39	3.85	17.73	34.5*	14.61	28.06*	11.52	2.89	12.89
Control A	35.05	-	30.05	-	3.27	-	30.1	-	25.16	-	2.56	-

* Significant at 0.05 level of LSD as compared to control.

GPE: Growth Promotion Efficiency.

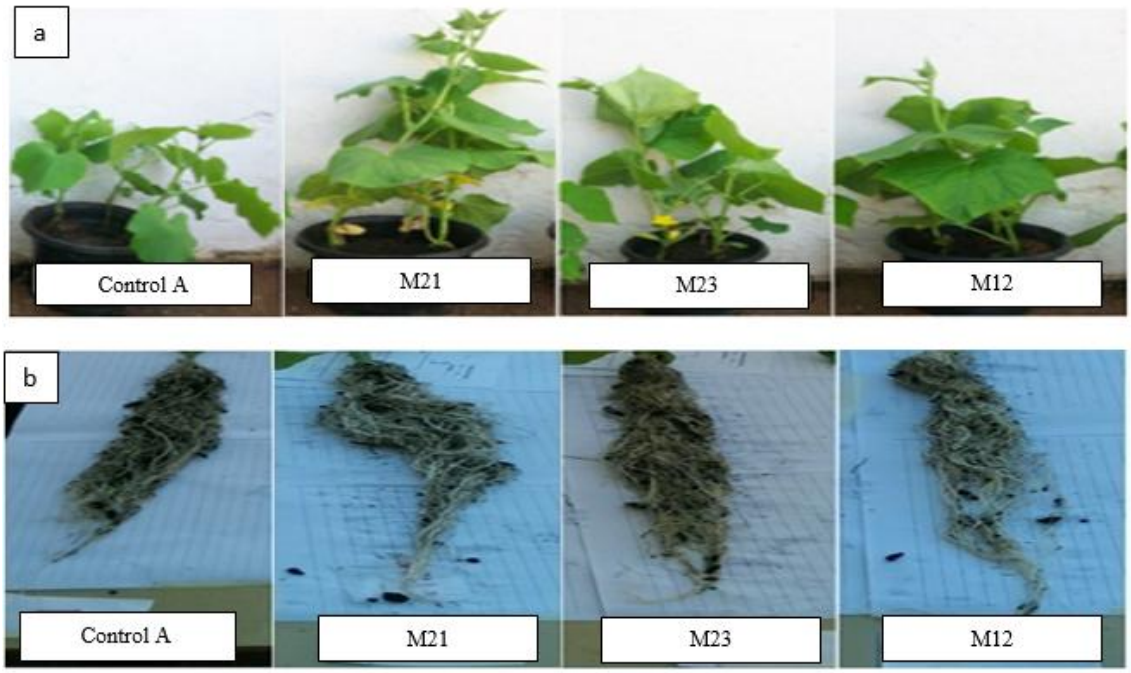

Figure 1: In Vivo, Efficacy of B*acillus* sp. on agronomic growth parameters.

a: Air parameters; b: Root parameters; Control A: not contaminated.

We notice that the *Bacillus* sp. M21, M23 and M12 led to a significant effect on the stimulation parameters of Cucumber plants growth compared to control (Table 1, Figure 1).

However, *Bacillus* sp. M21presents a highly significant effect on all growth parameters and the highest values of the growth promotion efficiency (GPE). It has a GPE (%) of 71.18; 62.79; 80.79, 81.51; 65.44 and 84.37 respectively for air length, root length, air fresh weight, fresh root weight, air dry weight and root weight.

While *Bacillus* sp. M23 and M12 have a significant effect on the stimulation parameters of Cucumber plants growth with the exception of air and root dry weight. These results are similar to those, which relate by Kidoğlu *et al*. (2008) and Ertan *et al.* (2015).[22, 23] These latest proved that inoculation with *Bacillus* sp. increased the growth parameters of Cucumber. Chun-Hao et *al.* (2015)[24] also found that *Bacillus amyloliquefaciens* stimulates growth parameters of watermelons compared to the control.

Several researchers have also reported that the use of *Bacillus sp* improved plant growth in different crops of vegetable such as Cucumber, arugula, lettuce, tomato, cauliflower, pepper, melon, watermelon, potato and radish.[25, 26, 27, 28, 29, 30, 31, 32]

Evaluation of *Bacillus* sp. ability on the reduction of Cucumber Fusarium wilt

The effect of *Bacillus* sp. strains on the reduction of Cucumber Fusarium wilt caused by *Fusarium oxysporum* presented in the table (2) and Figure (2).

Table 2: I*n Vivo* Efficacy of *Bacillus sp* on the growth parameters of *Cucumis sativus L.*in the presence of pathogen *Fusarium oxysporum*.

	Air Parameters			Root Parameters		
Treatment	**Length (cm)**	**Fresh weight(g)**	**Dry weight (g)**	**Length (cm)**	**Fresh weight(g)**	**Dry weight(g)**
M23+Fo	50*	50.67*	4*	46*	31.31*	3.51*
M21+Fo	49*	39.68*	2.53	40*	20.08*	1.83
M12+Fo	40*	19.99*	2.02	35*	12.85*	1.12
Control B	25	11.08	1.05	23	10.47	0.82

*significant at 0.05 level of LSD as compared to control.

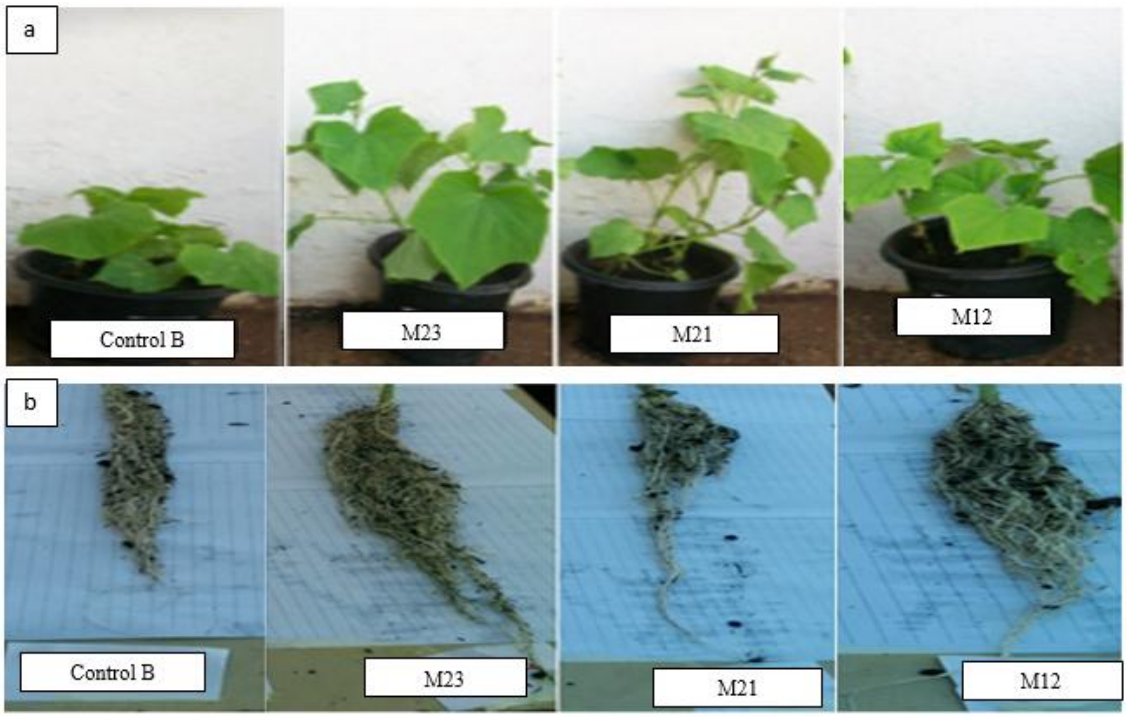

Figure 2: I*n Vivo,* Efficacy of *Bacillus* sp. on the reduction of Cucumber Fusarium wilt caused by *Fusarium oxysporum*. a: Air parameters; b: Root parameters; Control B: Only *Fusarium oxysporum*.

We notice that the inoculation of Cucumber seedlings with *Bacillus* sp. M21, M23 and M12 in the presence of *Fusarium oxysporum* led to a reduction of Cucumber Fusarium wilt symptoms as compared to the control (Table 2 and Figure 2).

Bacillus sp. M23 shows a highly significant effect on all growth parameters as compared to other *Bacillus* sp., respectively with an air maximum length (50.67cm), an air fresh weight (50g), an air dry weight (4g), a root length (46cm), a root fresh weight (31.31g), a root dry weight (3.51g). While *Bacillus* sp. M21 and M12 present a significant effect on agronomic parameters of the Cucumber with the exception of air and root dry weight.

Disease severity

The Disease incidence and biocontrol efficacy showed in Figure (3).

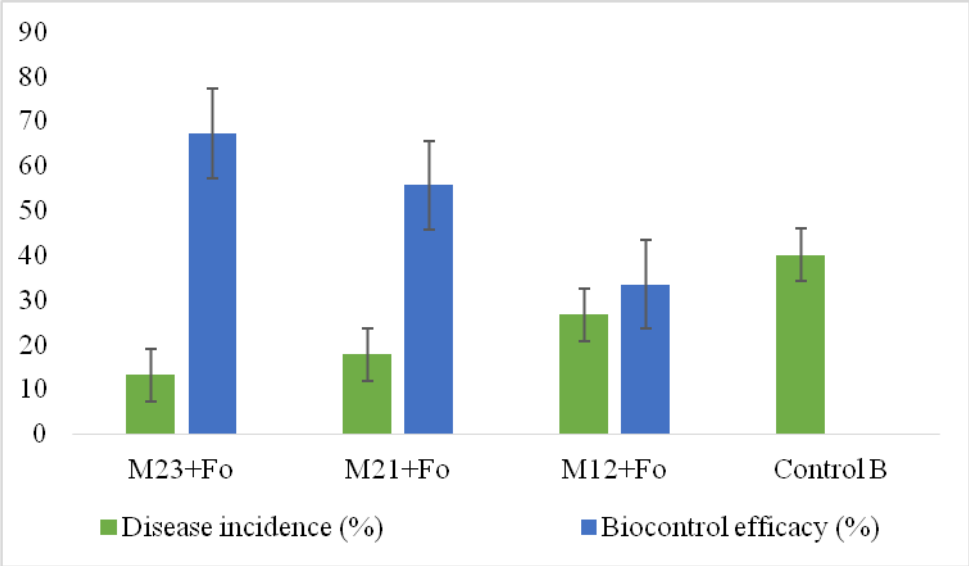

Figure 3: The disease incidence and of biocontrol treatments efficacy with *Bacillus* sp. against *Fusarium oxysporum in vivo.*

Bacillus sp M23 showed the lowest value of the disease incidence 13.11% and the highest value of biocontrol efficacy 67.22% against *Fusarium oxysporum*. However, *Bacillus* sp. *M12* has the highest incidence of the disease 26.66% and the lowest value of the biocontrol efficacy 33.35%.

Ahmed and al. (2009)[33] found that *Bacillus megtela* completely reduce the disease incidence followed by *Bacillus* sp. No2 and *Bacillus subtilis* No2 respectively with a biocontrol efficacy of 93.33%, 91.67%. Otherwise, several studies clearly showed that *Bacillus sp* significantly reduced disease incidence of Fusarium on Cucumber that concurred with our results.[34, 35, 36] Another study investigated the effect of *Pseudomonas* FR43 on the reduction of potatoes disease caused by *Fusarium oxysporum*. They showed through this study a significant reduction of the disease incidence with 15.3%, and significant biocontrol efficacy of 79.92% against *Fusarium oxysporum.*[37]

CONCLUSION

The isolated strains from plant roots of the Mint (*Mentha rotundifolia* L.) have a stimulatory effect on the growth parameters, and a protective effect against Fusarium wilt of Cucumber caused by *Fusarium oxysporum*. These two effects allow placing these *Bacillus sp* bacteria (M23, M21 and M12) among PGPR bacteria (Plant Growth Promoting Rhizobacteria). However, these strains may be involved in the biological elimination of pathogens.

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