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Efficacy of *Bacillus Subtilis* and *Bucillus Pumilus* Isolates on Linear Growth of *Pencelium Italicum* and *Pencelium Degitatum In Vitro*

Walid Ebrahim Mohammed Elash*

Plant Pathology Research Institute, Agricultural Research Center, 12619, Giza, Egypt

***Corresponding Author:** Walid Ebrahim Mohammed Elash, Plant Pathology Research Institute, Agricultural Research Center, 12619, Giza, Egypt.

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Source of bioagents

Tow bacterial bioagent isolate were kindly obtaind from kamhawy, 2012 namely, *B. subtilis* and *B. pumilus* were used in this study.

An in vitro experiment

An in vitro experiment was conducted to find out the antagonistic effect of tow bacterial isolates against *Pencelium italicum* and *Pencelium digitatum*, on PDA by dual culture technique using Noval ring method (Adetuyi and Cartwright 1985). A mycelial plug of *Pencelium*, was cut from colony margin by a 0.8 cm in diameter cork borer and placed onto the center of a Petri dish containing PDA medium. A cicular line, made with the edge of sterile 50 mm diameter glass tube dipped in suspension of tested bacteria (10^8 cfu/ml) and stamped on the medium surrounding the fungal inoculum. The bacterial suspension made according to the methods described by Prakong et al. (2004). Plates were incubated for 7 days at 28°C. Colony diameter of *Penclium* was measured and compared to control experiment where the bacterial was replaced by sterile distilled water. Four replicates used for each bacteria isolates. The results expressed as mean the percentage inhibition of the growth of the fungus using the following formula: fungal growth in control (mm)-fungal growth in treatment (mm) / fungal growth in control (mm) x 100.

Efficacy of biocontro agent (BCA) on Pencelium rot control of citrus fruits

Citrus fruits in post-harvest stage were used in this experiment. The fruits were disinfected with 70% alcohol and flamed before getting pierced with a sterilized needle to open 10 wounds on each fruit. Two experiments were separately performed. In the first experiment, 0.1 ml spore suspensions of *Pencelium italicum* and *Pencelium digitatum*, ($4x10^5$ spores /ml) was inoculated at the wounded areas of the citrus fruits, then incubated at room temperature for 24 h prior to the spray of cell suspension of bacterial antagonist (10^8 cfu/ ml). For the second experiment, citrus fruits were sprayed with the bacterial antagonist and kept for 24 h at room temperature before they were inoculated with *P. italicum* or *P. digitatum*, The fruits from two experiments were incubated at room temperature (27+2°C) before disease severity was observed and recorded. There were five replicates in each treatment. The disease severity was recorded after 10 days incubation by measuring rotted area using the following scale where 0=no lesion, 1= 1-10%, 2= 11-20%, 3= 21-30%, 4= 31-40, 5= 41-50%,6= 51-60%, 7= 61-70%, 8= 71-80%, 9= 81-90%, 10=91-100% fruit surface were diseased.

Results

The two biocontrol agent(BCA), *B. subtilis* and *B.pumauis* were tested for an antagonistic effect against *Pencelium italicum* and *Pencelium digitatum* in dual culture technique. The growth inhibition of *Pencelium italicum* and Pencelium degitatum by the two biocontrol agent was determined. As shown in figure (1), the two isolates of the biocontrol agent gave almost a total reduction of the growth of the two isolates of Penicillium in the laboratory after a weak of incubation. Yong Huang (2008). reported, with Bacillus showing strong antagonistic activity against *Penicillium digitatum*. B. (1.6×10^{10} to 1.6×10^{12}*cfu ml-1) gave significant control of *P. digitatum* infection

in Valencia orange, which was as effective as imazalil (500 µg ml-1) and was significantly better than benomyl treatment (500 µg ml-1). When lower concentrations of B. (1.9×10^7 to 1.9×10^9 cfu ml-1) were tested on Washington Navel orange and Lisbon lemon fruit, the antagonist caused significant control of P. digitatum infection at two inoculum levels (6.5×10^4 and 6.5×10^5 spores ml-5). Concentrations of both the pathogen and the antagonist affected the biocontrol effect. Rania Hammami et al.(2022) reported that overall, 180 yeasts and bacteria isolated from the peel of citrus fruits were screened for their in vitro antagonistic activity against *Penicillium digitatum* and *P. italicum*, causative agents of green and blue mold of citrus fruits, respectively. Three bacterial isolates were selected for their inhibitory activity on mycelium growth. The bacterial isolates were identified as *Bacillus amyloliquefaciens*, *B.pumauis* and *B. subtilis* isolates significantly reduced the incidence of decay incited by *P. digitatum* and *P. italicum* on 'Valencia' orange and 'Eureka' lemon fruits. Moreover, they were effective in preventing natural infections of green and blue mold of fruits stored at 4 °C. The antagonistic efficacy of the three isolates depended on multiple modes of action, including the ability to form biofilms and produce antifungal lipopeptides, lytic enzymes and volatile compounds.

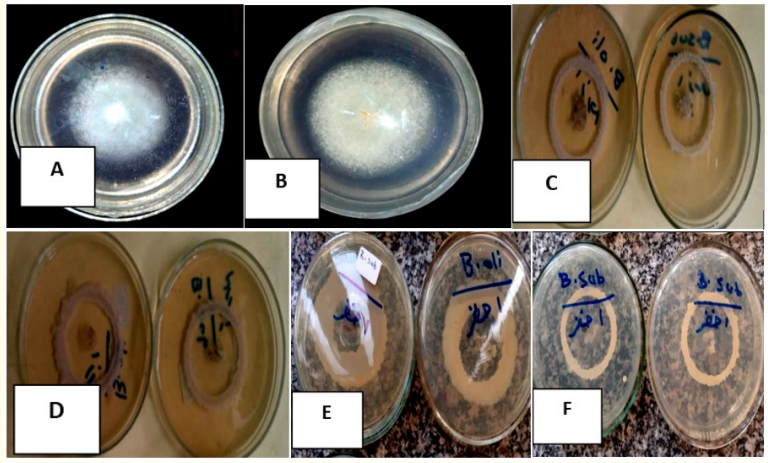

Figure 1: Novel ring bioassay show the highest percentage inhibition of *B. subtilis*_& *B. pumalus* isolates on *P. italicum* or *P. degitatum* After 10 days incubation. A&B: Control, C&D Bacillus subtilis. Fig., E, and F *Bacillus pumalis* isolates.

Efficacy of biocontro agent (BCA) on Pencelium rot control of citrus fruits.

Data presented in table 1 and fig. 2 clearly shows that, all promising isolates of bacteria could reduce disease severity on citrus fruits. It was noticed that, when bacterial antagonists were applied as spray application at 24 h before inoculation of *P. italicum* or *P. degitatum* was more efficient than after inoculation. *B. pumalus* provided the highest disease reduction percentage in both application were 79.96. and 75.28 % respectively of disease control.

Treatments	***Application treatment 24hr.before inoculation P. degitatum***		***Application treatment 24hr.after inoculation of P. italicum***	
	% Disease severity	***% Disease reduction****	***% Disease severity***	***% Disease reduction****
B.pumalis	10.20	79.96	22.00	75.28
Control(inoculated fruits, sprayed with water)	50	0.0	68.5	0.0
B. subtilis	30.00	53.12	32,00	46.66
Control(inoculated fruits, sprayed with water)	64.00	0.0	60.00	0.0
means	----------	74.36	----------	73.65
L.S.D. at %5 for Treatments(T) = ….., Spray(S)= ….., (T) x(S)= ….				
* disease reduction (%) recorded 10 days after inoculation.				

Table 2: Efficacy of bacterial antagonists for reducing *P. italicum* or *P. degitatum* rot disease on citrus plants when sprayed before or after treated *B. subtilis*_and *B. pumalus* for 24h.

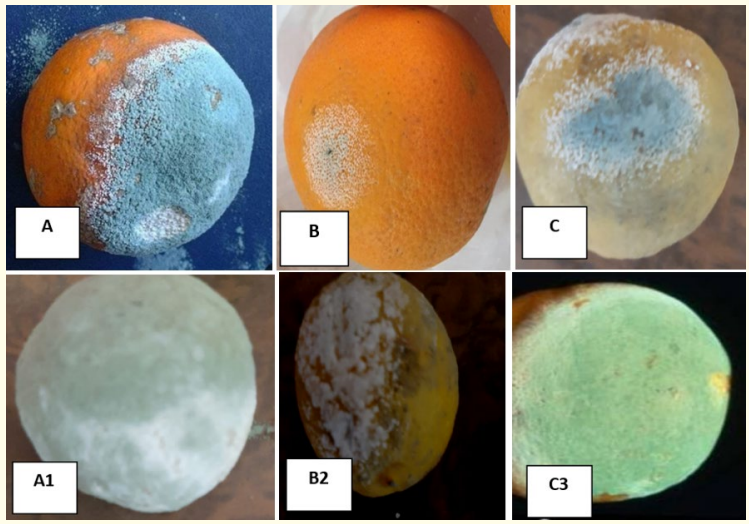

Figure 2: Efficacy of bacterial antagonists *B. subtilis*_and *B. pumalus* isolate for reducing *P. italicum* or *P. degitatum* disease on citrus fruits when sprayed before and after inoculation each pathogen for 24h (A) control treatment with water (B) *B. pumalus* treatment before inoculation of *P. Degitatum*. (C) *B. pumalus* treatment before inoculation of *P. Degitatum*. (A1) control treatment with water. (B1) *Bacillus subtilis* treatment before inoculation of P. italicum. (D) Bacillus subtilis. treatment after inoculation of *P. italicum*.

The antagonrstic activities of *Bacillus* spp. could be attributed to the production of endospores so, it more adapted to environmental extremes than the pathogen. Mechanism of biological control of plant pathogens generally involve competition for nutrients, production of bacterial metabolites such as iron chelating siderophores, hydrogen cyanide (HCN), antibiotics, extracellular lytic enzymes induced systemic resistance (O, Sullivan and O,Gara, 1992; Van Loon et al., 1998). O, Sullivan and O,Gara, 1992 reported that, successful bacterial antagonists often show a synergistic combination of mechanisms responsible for a successful antifungal.

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