Title Combining biocontrol agents to optimize the biological control of *Penicillium digitatum*

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Abstract

The green mold, caused by *Penicillium digitatum*, is responsible for huge losses of citrus fruits on the commercialization stage and it is considered limiting factor of production. The most effective method of controlling is the application of fungicides. However, concerns with human and environmental health about pesticide disposal residue levels on fresh commodities, and development of fungicide-resistant strains of pathogens have led to the search for new alternatives of control, among these the biological control. Four biocontrol agents, two yeast (*Saccharomyces cerevisiae)*, isolates CR-1 and K-1, and two bacteria (*Bacillus subtilis*), isolates BCAs 69 and 84, were tested separately and together for suppression of *P. digitatum*. The aim of the research was to determine whether the use of their combination would optimize the biological control and would reduce the variability of control efficacy under environmental condition would optimize the biological control and would reduce the variability of control efficacy under environmental condition (27°C and 70% RH). Fruits of acid lime 'Tahiti' were washed, surface sterilized with sodium hypochlorite to 0.7%, injured in two equidistant points, in the equatorial region of the fruit, with a stylus sterilized at a depth of 3 mm and inoculated with suspensions of conidia of *P. digitatum* (1×10^5 spores/mL) 24 hours before and after of the treatments. The suspensions of antagonists (1×10^8 cfu/mL) were applied separately or in mixture. Witnesses corresponded to the fruits inoculated and treated with fungicide (0.2% V/V); fruits inoculated and treated with water, fruit without inoculation and without treatment. For each test, a completely randomized design was used with three replications, with 15 fruits each. The evaluation corresponded to the percentage of healthy fruit. The result showed that the mixture K-q plus CR-1, provided 71% and 62% of healthy fruit, when the fruits were treated 24 h before and after of inoculation, respectively. Application of more then one biocontrol agent is suggested as a reliable means of reducing the variability and increasing the reliability of biological control.