Title *In vitro* activity of imazalil against *Penicillium expansum*: Comparison of the CLSI M38-A broth microdilution method with traditional techniques

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Abstract

Penicillium expansum is one of the most important pathogens that cause blue mold in stored apples and is regarded as the major producer of the mycotoxin patulin. Imazalil is one of the fungicides used in Spain to control postharvest blue mold, but development of fungal resistance has been reported in *P. digitatum* and *P. italicum*. The most common used methods to detect antifungal susceptibility of fungal crop pathogens *in vitro*, are direct-plating isolates in media amended with various concentrations of fungicide and determining inhibition of growth and/or spore germination. These techniques are time- and labor-intensive and are not suitable if a large number of isolates has to be evaluated. On the other hand, the broth microdilution method M38-A is the reference method developed by the Clinical and Laboratory Standards Institute (CLSI) for antifungal susceptibility testing in some clinical fungi, but *Penicillium* spp. are not included. Due to the lack of a standard method, the aim of this work is to evaluate the suitability of an adaptation of the CLSI M38-A method to monitor *P. expansum* susceptibility to imazalil in comparison with other techniques. A total of 128 *P. expansum* strains have been studied (118 isolates from apples and pears, 5 from grapes and 5 reference strains). Imazalil has shown to be highly active *in vitro* against all the *P. expansum* isolates tested, as all the evaluated parameters were in the range reported for imazalil sensitive *Penicillium* spp. The mean minimum inhibitory concentration determined by broth microdilution method and by agar dilution method (48–72 h readings) was 0.0625 μg/ml and 0.11–0.12 μg/ml respectively. The mean concentration that inhibited the size of colonies (48–72 h) and spore germination by 50% was 0.05–0.06 and 0.04 μg/ml respectively. Our results highlight that the broth microdilution method CLSI M38-A is a good alternative to be used in screening the *in vitro* activity of imazalil against a large number of isolates.