

Identification and Control of the Fungal Contaminants in *Vitro* Cultures of *Heuchera hybrida* (Coral Bells)

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Heuchera hybrida, also called "coral bells," is a versatile perennial with attractive foliage and bell-shaped flowers. To meet the increasing demand for commercial scale cultivation, tissue culture protocols have been developed. There is a necessity to suppress the growth of fungal contaminants that were present in the original explant or introduced as laboratory contaminants without causing an adverse effect on tissue-cultured plantlets. The goal of this study was to identify fungal contamination in *in vitro* propagated *H. hybrida* and control those using fungicides. Complete Randomized Design (CRD) was used to conduct the experiment. One-way, two-way (Analysis of Variance) ANOVA, and linear regression analysis methods were used. The contaminated fungi were isolated from tissue culture media and cultured on Potato Dextrose Agar and incubated at 25°C for one week. The identification of fungus were carried out by using macroscopic and microscopic examinations depending on the colony color, shape, hyphae, conidia, conidiophores and arrangement of spores. For the molecular identification of the contaminated fungus, the extracted fungal DNA was amplified by PCR using a specific internal transcribed spacer primer (ITS1 / ITS4). Six fungicides (Carbendazim[®], Topsin M 70[®], Chlorothalonil[®], Mancozeb[®], Antracol[®], and Homai[®]) were tested. The effectiveness of fungicides was evaluated using the inhibition zones produced by fungicides against fungal contaminants. Four different types of fungicides were chosen for *in vitro* screening and incorporated into the MS medium at rates of 75%, 50%, 25%, and 10% of its recommended dosage. Three fungal contaminants were identified as *Penicillium spp.*, *Phlebia acerina*, and *Cladosporium spp* based on both microscopic and macroscopic features and molecular confirmation. Topsin M 70[®] showed strong fungicidal effects on *Penicillium spp* and *Cladosporium spp.*, while having a fungistatic effect on *Phlebia acerina*. All the fungicide-treated samples did not have any fungal contamination during the multiplication period of *H. hybrida*. Topsin M 70[®] in tissue culture medium stimulated *H. hybrida* growth without causing visual toxicities in plantlets. The results of the experiment revealed that a 100 ppm concentration of Topsin M 70[®] effectively controlled the identified fungal contamination in *H. hybrida*, avoiding annual production losses due to fungal contamination.

Keywords: Fungal contaminants, Fungicides, *Heuchera hybrida*, *in vitro* propagation