2nd International Conference of Multidisciplinary Approaches (iCMA), 2015
Faculty of Graduate Studies,
University of Sri Jayewardenepura,
Sri Lanka
ISSN: 2386 – 1509

EFFECT OF INCORPORATION OF FUNGICIDES INTO TISSUE CULTURE MEDIUM ON FUNGAL CONTAMINANTS OF IN VITRO GROWN KAEMPFERIA GALANGA

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Kaempferia galanga is an important medicinal plant used in Ayurvedic medicine. To meet the increasing demand in Ayurvedic medicine, tissue culture protocols have been developed to produce healthy plants for mass propagation. Fungal contaminants growing on cultured tissues even after intense surface sterilization cause a great problem that adversely affects the survival of *in vitro* cultured tissues. There is a necessity to develop a method to suppress the growth of fungal contaminants without causing adverse effects on tissue cultured plantlets. In this study, rhizome buds were surface sterilized using the standard protocol and cultured on Murashige and Skoog (MS) basal medium supplemented with 3% (w/v) of sugar, 0.01% (w/v) of myo-inositol and 0.8% (w/v) of agar. Cultures were incubated at 25±1oC under 16 h light. Fungal contaminants from rhizome buds of *K. galanga* were isolated and identified using morphological characters. Four broad spectrum fungicides; Hayles-carbendazin, topsin M-70, dithane M-45 and coblite-copper oxychloride were tested for their effectiveness of inhibiting isolated fungi using well-diffusion method. Effectiveness of fungicides was evaluated using the inhibition zones produced by fungicides against fungal contaminants. The effective method of incorporating fungicide into tissue culture medium for inhibition was determined by performing three methods; incorporating fungicide in powdered form into MS molten medium, fungicide solution onto solidified medium and dipping the explants in fungicide solution at different time intervals. Minimum inhibitory concentration was determined incorporating different concentrations of the most effective fungicide into the medium. Observations were made after 60 days of incubation.

Five fungal isolates were identified as *Aspergillus niger*, three other *Aspergillus* species and *Rhizopus* species. Hayles-carbendazin exhibited the highest inhibition of fungal contaminants of tissue cultured plantlets. Incorporation of 750 ppm of Hayles-carbendazin powder into molten tissue culture medium prior to solidification was observed as the most effective method. Results reveal that incorporation of the fungicide Hayles -carbendazin into tissue culture medium is an effective method for suppressing fungal contaminations in tissue culture of *K. galanga*. However, the concentration of fungicide added to the medium markedly affects the growth of the plant tissue. As literature evident that contamination is one of major problems associated with *K. galanga* tissue culture and different surface sterilization methods used could not eliminate the fungal contamination, the results of this

study would be an important finding in eliminating the problem. However, results obtained suggest that use of suitable concentrations of fungicides is a prime importance in tissue culture of *K. galanga*.

Keywords: *Kaempferia galanga, Fungal contaminants, Hayles-carbendazin, Tissue culture, Fungicide concentrations*