SCREENING OF BIOFUNGICIDES TO CONTROL THE FUNGAL DISEASES CAUSED BY *Colletotrichum gloeosporioides*

By

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ABSTRACT

Fungal polyamines play a major role in controlling the growth and development of the fungal cells. Higher polyamine levels enhance the fungal growth whereas lower levels retard the growth. Therefore, depleting polyamine level by selective inhibition of poly amine biosynthesis through specific inhibitors could be used to control a variety of fungal diseases in plants. Following this line of thinking we aimed to identify potential and specific inhibitors of polyamine biosynthetic enzymes.

The fungus *Colletotrichum gloeosporioides* which causes anthracnose in a wide range of plants in many parts of the world was selected for the study. Arginine decarboxyalse (ADC) and Ornithine decarboxylase (ODC) are the rate limiting enzyme of the polyamine biosynthesis. ADC was isolated from *Colletotrichum gloeosporioides* and purified 37.56 fold with 37.04% recovery. Formation of the product was continued over 20 minutes and found linear for an initial period of 10 minutes. Optimal assay temperature for enzymes lied between 35 °C to 45 °C with a maximum at 40 °C and optimum pH is found to be at pH 5.2. According to the results, no evidence for ornithine decarboxylase activity was detected.

Several plant extracts which posses antifungal activity were tested as inhibitors for the enzyme. From the tested plant extracts, aqueous extract of *Allium sativum* (Garlic), *Ocimum sanctum* (Maduru thala), *Ocimum gratissimum* (Gas thala), *Jatropha curcas* (Wata Endaru) and methanolic tuber extracts of *Acorus calamus* (Wada Kaha), *Zingiber zerumbet* (Wal inguru), *Curcuma zedoaria* (Haran Kaha) were found as inhibitors for the enzyme (ADC) whereas aqueous leaf extract of *Vitex negundo* (Nika) and methanolic tuber extract of *Costus speciosus* (Thembu) were found as non inhibitors for the enzyme. Plant extracts which showed inhibitory effects against ADC activity were screened *in vitro* against mycelial growth. All the plant extracts showed inhibitory effects against mycelial growth of the fungus.

Minimum inhibitory concentration (MIC) values for *Allium sativum*, *Ocimum sanctum*, *Ocimum gratissimum* and *Jatropha curcas* were 0.40 g/ml, 0.65 g/ml, 0.70 g/ml and 0.90 g/ml, respectively. Minimum lethal concentration (MLC) values for *Allium sativum*, *Ocimum sanctum*, *Ocimum gratissimum* and *Jatropha curcas* were 0.45 g/ml, 0.70 g/ml, 0.80 g/ml and 0.90 g/ml, respectively. MIC values for *Acorus calamus*, *Zingiber zerumbet* and *Curcuma zedoaria* were 2.2 mg/ml, 2.4 mg/ml and 3.4 mg/ml, respectively. MLC values for *Acorus calamus*, *Zingiber zerumbet*, and *Curcuma zedoaria* recorded as 2.4 mg/ml, 2.8 mg/ml and 3.6 mg/ml, respectively. Among the tested plant extracts, aqueous extracts of *Allium sativum* and methanolic tuber extract of *Acorus calamus* showed a remarkable inhibitory effect against enzyme activity and subsequent mycelial growth.

Aqueous plant extracts were sequentially extracted into hexane, dichloromethane and ethyl acetate, respectively. The promising dichloromethane and ethyl acetate fractions of *Ocimum sanctum*, *Ocimum gratissimum*, *Jatropha curcas* and *Allium sativum* were subjected to TLC analysis and tested for antifungal activity. Dichloromethane extracts of *Ocimum sanctum*, *Ocimum gratissimum*, *Jatropha curcas* and *Allium sativum* produced inhibition zones at the R_F values of 0.82, 0.83, 0.84, and 0.63, respectively in the *Colletotrichum gloeosporioides* preparative TLC plates. Ethyl acetate extracts of *Ocimum sanctum*, *Ocimum gratissimum*, *Jatropha curcas* and *Allium sativum* produced inhibition zones at the R_F values of 0.83, 0.82, 0.81 and 0.62, respectively. Values were similar to the R_F values obtained during TLC separation of sequentially extracted above fractions. Results revealed that the compounds which account for inhibitory action towards mycelial growth were located at R_F values of 0.82, 0.83, 0.84, and 0.63 in dichloromethane fraction where in ethyl acetate fraction, they were located at R_F values of 0.83, 0.82, 0.81 and 0.62 for *Ocimum sanctum*, *Ocimum gratissimum*, *Jatropha curcas* and *Allium sativum*, respectively. In methanolic tuber extract of *Acorus calamus*, the active constituent was located at R_F 0.71. Results suggest that aqueous extract of *Allium sativum*, *Ocimum sanctum*, *Ocimum gratissimum*, *Jatropha curcas*, and methanolic tuber extracts of *Acorus calamus*, *Zingiber zerumbet*, and *Curcuma zedoaria* are potential candidates plants for the management of *Colletotrichum gloeosporioides* through inhibition of polyamine biosynthesis enzymes.