Use of molecular features for identification of isolated fungal pathogens of big onion damping off disease and *Trichoderma* spp. isolated from soil

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Big onion (*Allium cepa* L.) is one of the economically important spices grown in Sri Lanka. Damping off disease caused by *Fusarium* sp. during nursery stage of growth poses a major factor that affect the yield significantly. Application of fungicides decrease incidence of damping off disease considerably, but this is neither economical nor environmental friendly. Thus, disease management practices have to be directed towards biological control strategies. *Trichoderma* spp. have been extensively studied as biological control agents for controlling numerous soil-borne fungal pathogens. In the present study, isolation and identification of fungal pathogens associated with damping off disease of onion and *Trichoderma* spp. present in soil of the same onion fields was carried out with a view to using the *Trichoderma* spp. in the management of damping off pathogens. Pathogens associated with damping off were isolated from diseased and healthy seedlings (7-30 days old) collected from the fields in the Matale and Anuradhapura districts. Seedlings were surface sterilized and plated in Potato Dextrose Agar (PDA) supplemented with tetracycline. Soil samples collected from the same fields were used for the isolation of *Trichoderma* spp. using the Warcup method. Based on morphological characteristics and using identification keys, the fungal pathogens isolated from seedlings were identified as *Fusarium, Curvularia, Alternaria* and *Sclerotium* spp. and 14 fungal species isolated from soil samples were identified as *Trichoderma* spp. Although fungi can be identified using morphological features, the use of molecular biological methods tend to be more accurate. Therefore, the identity of isolated fungal species was confirmed by molecular biological methods. Genomic DNA of *Fusarium* spp., *Alternaria* spp., *Trichoderma* spp. were extracted. Molecular characterization of these DNA was carried out using Polymerase Chain Reaction (PCR) where the Internal Transcribed Spacer (ITS) region of rDNA gene was amplified using ITS-1 and ITS-4 primer pairs. The products were subjected to agarose gel electrophoresis. The procedures were repeated 3 times. Results showed 550 bp size bands characteristic of *Fusarium* spp. and 570 bp products specific to *Alternaria* spp. confirming the previous identity using culture based methods. Fungal species isolated from soil showed products of 600 bp which corresponds to *Trichoderma* sp. Molecular characterization of the potential biocontrol agents *i.e. Trichoderma* spp. and *A.cepa* L. pathogens using PCR amplification of ITS region confirmed the preliminary identities carried out using culture based methods.

Key words: Trichoderma spp., Fusarium spp., ITS, PCR, A.cepa L.

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