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Original Article

Trichoderma Species Associated with Medicinal Plants

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ABSTRACT

Objective: The genus *Trichoderma* comprises a great number of fungal strains that act as biological control agents, the antagonistic properties of which are based on the activation of multiple mechanisms. **Methods:** Trichoderma strains exert biocontrol against fungal phytopathogens either indirectly, by competing for nutrients and space, modifying the environmental conditions, or promoting plant growth and plant defensive mechanisms and antibiosis, or directly, by mechanisms such as mycoparasitism. In order for identification of *Trichoderma* species, sampling was performed from plant tissues and soil samples of medicinal plants in the South Khorasan province during 2012-2114 years. Several isolates of *Trichoderma* were isolated from soil and tissue samples using the *Trichoderma* selective medium. **Results:** Isolates were identified based on macroscopic and microscopic morphological characteristics such as a growth and shape of the colony, type of conidiophore formation, shape and size of phialide and conidium on CMD and MEA and using valid *Trichoderma* keys. So far, four species were identified harzianum, crassum, brevicompactum, and virens spices. *T. harzianum* was the most frequent species among isolates and isolated from Russian olive, Camelthorns, Jujube, Barberry and Basil samples.

1.INTRODUCTION

Trichoderma species, which present in manure, soil and decaying plant tissues, are avirulent plant symbionts and parasites of other fungi (Kubicek et al., 1998). Their dominance in soil may be attributed to their diverse metabolic capability and aggressive competitive nature(Harman, 2006). *Trichoderma* controls phytopathogen with secretion antibiotics and fungal cell wall degrading enzymes, out compete pathogenic fungi for nutrients and mycoparasitism mechanisms. (Chincholkar and Mukerji, 2007). More than 1700 species of medicinal and industrial plants are known in Iran which only 200-300 species are harvested. Others spontaneously grow, dry and die without any use(Amin, 1991). The aim of this study was to identify and characterized *Trichoderma* spices associated with medicinal plants in South Khorasan province for improve the production and quality of medicinal plants.

2. MATERIALS AND METHODS

Soil samples were collected from different regions of South Khorasan province in the east of Iran during 2012-2014 years. Trichoderma strains were isolated from soil samples by first washing the soil using sterilized water, 1/10,000 dilutions of the samples were prepared, and then followed by plating 1 mL of the soil dilutions on potato-dextrose agar (PDA; 20 g white potato boiled and filtered, 20 g dextrose, 20 g agar, 1,000 ml distilled water) in a 9-cm-diam petri plate at 25°C in darkness

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until the mycelium covered the entire plate; in order to suppress bacterial growth, 30 mg/L of streptomycin was added(Panda et al., 1989). Growth rates were determined on PDA, cornmeal dextrose agar (CMD; Difco cornmeal agar + 2 %w/v dextrose) and a defined, low nutrient agar (SNA) at 25 and 35°C in darkness for 72 h. All isolates were grown on PDA, CMD and SNA in Petri dishes, incubated for 7 d at 25°C in the darkness, and then exposed to artificial light to stimulate conidium formation(Park et al., 2006). Morphological features of colonies were based on observations on CMD under the above growth conditions. Microscopic observations and measurements were made from preparations mounted in 3 % KOH. The statistics presented here are based on measurement of 50 mature conidia (± S.D.) and 30 phialides (± S.D.) at 100×magnification. To assess and describe their structure and morphology, conidiophores were taken from the edge of conidiogenous pustules or fascicles. Conidia were studied after 7 d of incubation(Rifai, 1969).

3. RESULTS

Sixteen isolates belonging to the *Trichoderma* genus were found and identified by macroscopic and microscopic characteristics of the colonies. This strain was distributed along with eight of the twenty sampled localities in South Khorasan (AmirAbad, Mohammad Shahr, Chahkand, Sarbishe, Nehbandan, Darmiyan, Cain and Aliabad). Nine isolates with effused conidiation, verticillate conidiophores with short side branches, ampulliform to lageniform phialides, subglobose to obovoid small conidia, fast growing and subhyaline to pale green colonies were identified as *T. harzianum* Rifai. This species was recovered from soil and tissue samples of Russian olive, Camelthorns, Jujube, Barberry and Basil. Several isolates of a rapidly growing *Trichoderma* species with effused conidiation, flat pustules concentrated near the margin of the colony, irregularly macronematous conidiophores branching, lageniform to ampulliform Phialides, broadly ellipsoidal to obovoid conidia were identified as *T. virens*. This species isolated from Russian olive, Jujube and Barberry samples. Isolates with rapidly growing green colonies, compact conidiation, yellowish pigmentation in agar and ellipsoidal dark green conidia were grouped in *T. crassum* Bissett. This species isolated only from Jujube samples. *T. brevicompactum* isolates from Barberry were characterized by a pachybasium-type morphology, subglobose conidia, fast growing green colonies, broad compacted zones sporulating, densely aggregated conidiophores in coalescent pustules, ampulliform short fields with Subglobose conidia.

4. DISCUSSION

South Khorasan province, located in the east of Iran, has a dry climate with significant difference between day and night temperatures. This province has a cold desert and half-desert climate with hot summers and cool winters and low recipitation(Russell and Cohn, 2012). Medicinal plants including jujube, barberry and saffron is planted in this province(Pooyan, 1989). During this research, many samples were taken from medicinal plants and *Trichoderma* spicese only were recovered from Russian olive, Camelthorns, Jujube, Barberry and Basil samples. *Elaeagnus angustifolia*, commonly called Russian olive is a species of *Elaeagnus*, native to Russia, Kazakhstan, Turkey and Iran. In Iran, the dried powder of the fruits is used mixed with milk for rheumatoid arthritis and joint pains(Aynehchi et al., 1982). Phytopthogenic fungi cause leaf spots,verticillium wilting and browning of leaves on Russian-Olive(Katz and Shafroth, 2003). Harzianum and virens spicese of *Trichoderma* isolated in this research can be used as a potent biocontrol agents for Russian olive disease control.

Alhagi, camelthorns or manna, is a genus of Old World plants in the family Fabaceae. Khan (2009) based on ethno-veterinary specifies of some medicinal plants in Pakistan, had stated that Alhagi eas used in care of gastrointestinal impaction of domestic animals. The application of *T. harzianum* strains which isolated in this research from AmirAbad region can increases the number of deep roots, thereby increasing the plant's ability to resist drought. Jujube (*Ziziphus jujube*) is a delicious fruit and an effective herbal remedy. It aids weight gain, improves muscular strength and increases stamina. In Chinese medicine it is prescribed as a tonic to strengthen liver function. Japanese research has shown that jujube increases immune-system resistance(Cheng et al., 2000; Ahmad and Beg, 2001). Several kinds of key pathogens such as *Alternaria alternata*, *Phoma destructiva*, *Fusicoccum* sp., *Valsa ceratosperma* (anamorph *C. sacculus*) and *Fusarium solani* were isolated from Jujube(Petri, 1940; Mirzaee et al., 2011; SHA et al., 2009; Saleh et al., 2012). The application of *Trichoderma* spiceses which isolated in this research from Jujube can reduce disease that caused by this pathogens.

Barberry (*Berberis vulgaris* L.) is a shrub native to central and southern Europe, northwest Africa and western Asia; it is also naturalised in northern Europe, including the British Isles and Scandinavia, and North America. Zereshk is the Persian name for the dried fruit of Berberis spp. specially that of B. integerrima, which is widely cultivated in Iran(Tehranifar, 2002). *Colletotrichum gloeosporioides*, *C. acutatum*, *Pestalotiopsis* sp., *Phomopsis* sp., *Sclerotinia sclerotiorum*, and *Alternaria* spp. were found to be associated with stem, leaf, flower and fruit disease of barberry(Waipara et al., 2005; Jahani et al., 2013; Stakman and Fletcher, 1930). The application of Trichoderma species which isolated in this research can control foliar and soil borne pathogenes in Barberry. Basil(*Ocimum basilicum* L.) is cultivated in large quantities in different regions of Iran(Sajjadi, 2006). Recently, there has been much research into the health benefits conferred by the essential oils found in basil.

Scientific studies in vitro have established that compounds in basil oil have potent antioxidant, antiviral, and antimicrobial properties, and potential for use in treating cancer(Stakman and Fletcher, 1930; Gülçin et al., 2007). *T. harzianum* isolates may induce systemic resistance, increased nitrogen use efficiency and increasing the number of deep roots in Basil plants. *T. harzianum* has been reported from all the geographical regions of the world on a wide range of crop plants (Kubicek et al., 1998). The use of *Trichoderm*a products control diseases and enhance growth of crops as a short term effects and reduce pesticide application as a long term effects. Presently, Trichoderma-based products are considered as relatively novel biological control agents which can help farmers reduce plant diseases and increase plant growth.

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