SHORT COMMUNICATION

Antifungal activity of the clove essential oil from *Syzygium aromaticum* on *Paecilomyces variotii* agent of pistachio dieback

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Abstract

Pistachio dieback is a serious disease in Rafsanjan (Iran) farms caused by *Paecilomyces variotii*. The aim of the present study was to investigate fungicidal effects of essential oil of cloves on *Paecilomyces variotii* agent of pistachio dieback. In order to evaluate the antifungal properties of *Syzygium aromaticum*, essential oil from leaf and bud of clove were obtained from hydrodistillation. The isolates were cultured in sabouraud dextrose agar for 48 h at 25°C. The concentration of 100, 200 and 400 ppm of the oil was used. Every day a diameter of fungal growth on culture medium was measured using a calibrated microscope. The lowest fungal growth was observed at concentrations of 400 ppm oil (1.421 Cm). Maximum fungal growth was observed in the control sample (6.1 Cm). Based on results of this study, it is recommended that the concentration of 400 ppm clove oil is used to reduce the growth rate of the *Paecilomyces*.

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Introduction
Pistachio dieback is a serious disease in Rafsanjan (Iran) farms caused by Paecilomyces variotii. Contamination of pistachio trees has been estimated ranged from 0-85%. On average, 17% of orchards in Rafsanjan are infected. Symptoms include dieback of twigs and branches, bark necrosis and pruning wounds on trees. Necrotic area has progressed slowly down branches and twigs covered round. It creates a gap in the branches and even in tree crown. Infected areas appear as dark bands. Necrotic area is mostly in surface and does not reach the inner part of the tree. Paecilomyces variotii is a common environmental mold that is widespread in composts, soils and food products. It is known from substrates including food, indoor air, wood, soil and carpet dust. Paecilomyces variotii is the asexual state of Byssochlamys spectabilis, a member of the Phylum Ascomycota (Family Trichocomaceae) (Houbreken et al., 2008).

In nature, essential oils have an important role in protecting plants. They serve as antibacterial agents, antiviral, antifungal, and insecticides, and also against the action of herbivores. Syzygium aromaticum (L.) Merr and Perry commonly called clove, belongs to the family Myrtaceae. Clove bud oil has biological activities, such as antibacterial, antifungal, insecticidal and antioxidant properties and antimicrobial material in food (Lee and Shibamoto, 2001). The high levels of eugenol contained in clove essential oil responsible for strong antimicrobial activity (Briozzo, 1989). Several constituents of clove has been identified, mainly eugenol, eugenyl acetate, beta-caryophyllene, 2-heptanone (Chaieb et al., 2007b), acetyleugenol, alpha-humulene, methyl salicylate, isoeugenol, methyleugenol (Yang et al., 2003), phenyl propanoines, dehydroeugenol, trans-confrireryl aldehyde, biflorin, kaempferol, rhamnocitrin, myricetin gallic acid, ellagic acid and oleanolic acid (Cai & Wu, 1996). The main constituents of essential oil are phenylpropanoines such as carvacrol, thymol, eugenol and cinnamaldehyde (Chaieb et al., 2007a). Several studies have demonstrated potent antifungal effects of clove (Arina & Iqbal, 2002; Giordani et al., 2004; Pawar & Thaker, 2006; Park et al., 2007). There has been no report of antifungal effects of essential oil of cloves on the Paecilomyces variotii. The aim of the present study was to investigate fungicidal effects of essential oil of cloves on Paecilomyces variotii agent of pistachio dieback.

Materials and methods
The fungal obtained from Biotechnology Institute of Scientific and Industrial Research Organization of Iran. The isolates were cultured in sabouraud dextrose agar for 48 h at 25°C. Essential oil from leaf and bud of clove were obtained from hydrodistillation. About 100 g of the powdered tissue was extracted using a Clevenger apparatus through water distillation for 3 hours. The essential oil, distilled using sodium sulfate, and kept at 4 °C until it was injected into GC. The yield of the oils was calculated based on dried weight of plant materials.

The concentration of 100, 200 and 400 ppm of the oil was used. Every day a diameter of fungal growth on culture medium was measured using a calibrated microscope. Fungus growth at each concentration was compared with control. The statistical design used was a split plot. Experiment was done in a completely randomized design with 3 replicates. The main plot involves different concentrations of essential oils in four levels and sub-plots, includ the 12 level (the first day to the twelfth day), respectively.

All the data were statistically evaluated using ANAVA followed by Dunn’s post-hoc multiple comparison test when the significance value is <0.05 using the same significance level. The criterion for statistical significance was taken as \( P < 0.05 \).

Results and discussion
Results of this study showed that clove oil reduced fungal growth. The antifungal activities of clove oil against Paecilomyces variotii at concentrations of 100ppm and 200 ppm were not significant in the first and second day. The lowest fungal growth was observed at concentrations of 400 ppm oil (1.421
Maximum fungal growth was observed in the control sample (6.1 Cm). Based on results of this study, it is recommended that the concentration of 400 ppm clove oil is used to reduce the growth rate of the *Paecilomyces* (Fig. 1, 2). Clove represents one of the major vegetal sources of phenolic compounds as flavonoids, hydroxibenzoic acids hidroxicinamic acids and hidroxiphenyl propens (Shan *et al*., 2005). With regard to the phenolic acids, gallic acid is the compound found in higher concentration. Flavonoids as kaempferol, quercetin and its derivates (glycosilated) are also found in clove in lower concentrations. The antimicrobial activities of clove have been proved against several bacterial and fungal strains (Francisco Cortés-Rojas *et al*., 2014). Sofia *et al*., (2007) tested the antimicrobial activity of different Indian spice plants as mint, cinnamon, mustard, ginger, garlic and clove. The only sampled that showed complete bactericidal effect was the aqueous extract of clove at 3%. At the concentration of 1% clove extract also showed good inhibitory action. Rana *et al*. determined the antifungic activity of clove oil in different strains and reported this scale of sensibility *Mucor* sp.>*Fusarium moniliforme* NCIM 1100>*Trichophyum rubrum*>Aspergillus sp.>*Fusarium oxysporum* MTCC 284 (Rana *et al*., 2011).

The chromatographic analyses showed that eugenol was the main compound responsible for the antifungic activity due to lysis of the spores and micelles. A similar mechanism of action of membrane disruption and deformation of macromolecules produced by eugenol was reported by Devi *et al* (2010). The anticandidal activity of eugenol and carvacrol has been reported by Chami *et al*., (2004). On the basis of the data presented, the essential oil of *Syzygium aromaticum* and its component have antifungal activity on *Paecilomyces variotii*.

![Fig. 2 Fungal growth after 7 days in different concentrations of clove oil.](image)

**Fig. 1** The interaction between the oil and the fungi growth (Mean± SE)

The references are as follows:

**References**


