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Antifungal Activity of Essential Oils from Some Medicinal Plants against Green Mold (*Penicillium digitatum*)

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Abstract: *In this study; screened five essential oils for fungitoxic effect against Penicillium digitatum that infect orange fruits. The five essential oils were commercial products derived from clove, thyme, sesame, black cumin and cinnamon. Antifungal activity of essential oils was compared by poisoned agar technique. In laboratory; among essential oils clove was the most effective showed 100% inhibition of mycelial growth at 1% concentration. Less antifungal effect was found with essential oils from cinnamon and thyme, while the oils from black cumin and sesame manifested no inhibition. Microscopic study on effect of essential oils on spores of P. digitatum showed distortion and shrinkage. Some essential oils might be promising source of a biological fungicide, applied directly before harvest or during postharvest.*

Keywords: *Essential oils, Antifungal, Penicillium digitatum, Biological control.*

1. INTRODUCTION

Fruits and vegetables are often subject to varying levels of microbial decay during the growing season, harvesting, handling, transport and post-harvest storage and marketing conditions, or after purchasing by the consumer. Fruits contain high levels of sugars and nutrients, and their low pH makes them vulnerable to fungal decaying (Singh and Sharma 2007). Pathogenic fungi usually infect the host through wounds and cause significant economic losses in the commercialization stage (Gatto *et al*., 2011).

Green mold decay caused by the fungus *Penicillium digitatum* is a serious worldwide problem in citrus fruit and production (El-Gali, 2014; 2016 a). The use of manufactured chemicals as fungicides is a main method to prevent or delay diseases and the postharvest rot is well known. Outspread use of fungicides has significant disadvantage including increased cost, worry about fungicides residues on crop, as well as risk for human health and environment (Nikos and Costas, 2007). The developments of alternative non-synthetic chemical strategies, which better comply with organic food standards, are needed. Among the various strategies proposed, biological control using natural products such as plant extracts and essential oils show most promise.

Many plant essential oils and their volatile constituents have been reported to possess potent antifungal activities. Essential oils are represent a defence mechanism against pathogens and pests, produced in different plant section and they also have been shown to own antimicrobial and antifungicidal properties (Znini *et al*., 2011). Several studies have investigated the antifungal properties of essential oils against postharvest pathogens (Anjum and Akhtar, 2012 ; Jhaleger et al., 2015 ; Wang et al., 2017). For example, Vitoratos et al. (2013) recorded inhibition in mycelial growth of *Botrytis cinerea*, *P. digitatum* and *P. italicum* under effect of plant essential oils in culture plates. The rosemary leaves oil has reported to be effective against *P. digitatum* in vitro (Handel et al., 2016). In the other study, Louhibi et al. (2016) recently reported the effective inhibition of the green mold fungus using essential oils derived from *Lavandula hybrida* and *Artemisia herba alba*. In this study, the essential oils derived from four medicinal plants (clove, cinnamon, thyme, sesame and black

cumin) were evaluated for efficacy against *Penicillium digitatum* under a modified atmosphere environment *in vitro*.

2. MATERIALS AND METHODS

Pathogen

Isolate of *P. digitatum*, was originated naturally infected sour orange fruits and maintained on potato sucrose agar(PSA) at 4 °C. Fresh cultures of the fungus were prepared by subculture of mycelia onto new PSA plate and the incubated at 23 °C for 3-5 days.

Oils

Commercial essential oils of plants were including clove (*Syzygium aromaticum*), cinnamon (*Cinnamomum aromaticum*), sesame (*Sesamum indicum*), black cumin (*Nigella sativa*) and thyme (*Thymus vulgaris*) and were obtained from Alexandria Company for Medicinal Industries.

Growth study

In the growth study, different essential oils were incorporated with potato agar medium in 1% concentration The *P. digitatum* was allowed to grow in the above test and control media. The diameter of the colony in both were measured and the percent inhibition to growth was deduced

using the following formula.

$$\text{Percent inhibition} = \frac{a-b}{a} \times 100$$

where 'a' is the radial growth of the colony in the control medium and 'b' the radial growth in the test medium.

Microscopic study of effect of EO on fungal mycelium and spores

Fifty microliters of EO were put on the center of the glass slides. Fresh fungal mycelium piece bearing spores was taken out of petriplate with inoculating needle and immersed in the drop of clove oil on slides. After 1h of incubation at ambient temperature, the slides were examined under light microscope and photographed.

Statistical analysis

Data were converted by using the formula Arc sin _x (Yazdi Samadi et al, 1997). Statistical analysis was performed with COSTAT and chart was drawn by Excel 2010 software.

3. RESULTS AND DISCUSSION

The effect of some essential oils on mycelial growth of *P. digitatum* was presented in Fig. 1. The results indicated that, the essential oils gave different effectives against fungal growth. Inhibition zone diameters of *P. digitatum* was completely inhibited by exposure to clove oil at 1%, while the oils from black cumin and sesame manifested no inhibition. The effect of several plant species essential oils was investigated by Abd-alla et al. (2014) ; El-Gali (2016 b) and Pandey et al. (2017). The pathway of activity of these complexes against fungi is unidentified but may be interrelated to their general capability to soften or otherwise dislocate the reliability of cell membranes and cell walls (Isman and Machial, 2006).

Clove oil could completely stop fungal growth. The results of thyme oil was followed by cinnamon oil, whose lowest showed 28.2% and 24.6% mycelial growth inhibition respectively. No effect was recorded at used black cumin oil, it was enhancing mycelia growth. Several studies reported that clove oil was most effective against radial fungal growth (Anjum and Akhtar, 2012 ; Hamini-Kadar, 2014 ; El-Gali. 2016). Cox et al. (2000) reported that tea tree oil inhibited respiration and increased the permeability of cell cytoplasmic and plasma membranes.

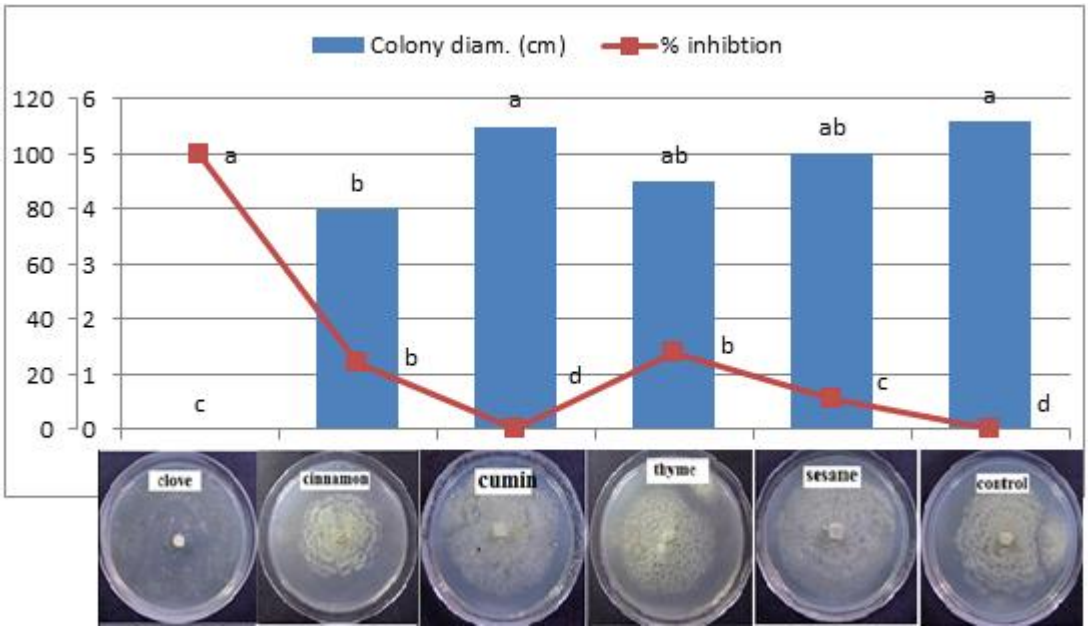

Figure 1. *Antifungal activity of 5 essential oils against mycelial growth of P. digitatum on PSA medium. (Columns followed by the same letter(s) are not significantly different at P= 0.05)*

In addition to all oils were changed in spores morphology. After 1 hr of treatment of fungal bearing spores with essential oils, lysis and distortion of spores was observed in the fungal spores (Fig. 2). The effect was profound and clearly visible in form of deformities and retraction of spore structure, such as thickness in cell wall and renal or lunar shape were observed. Microscopic study on effect of clove oil on spores of *Mucor* sp. and *M. gypseum* showed distortion and shrinkage (Rana, et al., 2011). Park et al. (2007) studied effect of clove oil on *Trichophyton mentagrophytes* with the help of transmission electron microscope. They observed the expansion of its endoplasmic reticulum near the cell membranes of a hyphal specimen treated with eugenol. In addition, the inner mitochondrial membranes were partially destroyed, with complete destruction of the cell wall. The accumulation of hydrocarbon molecules resulted in swelling of the membrane bilayer, as assessed by the release of fluorescence self-quenching of fluorescent fatty acid and phospholipid analogs. These effects on the integrity of the membrane caused an increased passive flux of protons and carboxyfluorescein. The effective concentrations of the different cyclic hydrocarbons correlated with their partition coefficients between the membrane and aqueous phase. The impairment of microbial activity by the cyclic hydrocarbons most likely results from hydrophobic interaction with the membrane, which affects the functioning of the membrane and membrane-embedded proteins (Sikkema, et al., 1994).

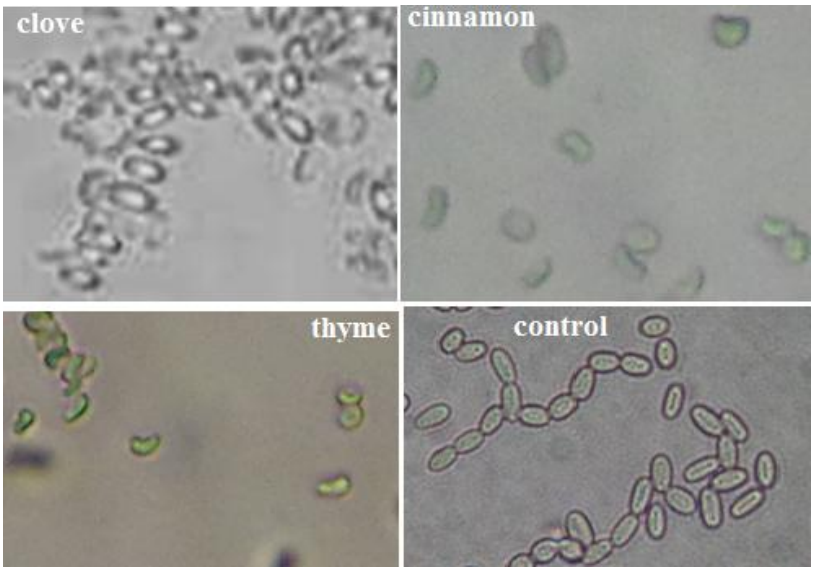

Figure 2. *Abnormal spores of P. digitatum treated with essential oil compared with the untreated (Con.).*

4. CONCLUSION

In conclusion this preliminary study showed that the essential oils derived from clove, cinnamon and thyme maybe used as alternative for the control of green mold on postharvest organic fruits especially as a natural fumigant in closed container or packaging.

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