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# Evaluation of lemongrass, thyme and peracetic acid against gray mold of strawberry fruits

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The efficacy of the essential oils, *i.e.* lemongrass and thyme oils as well as preacetic acid either as vapor or liquid phases against strawberry gray mould incidence, *Botrytis cinerea in-vitro* and *in-vivo* conditions. *In-vitro*, the obtained results indicated that all vapor treatments significantly reduced the linear growth and spore germination of *B. cinerea*. It was observed that rising concentration of tested chemicals reflected negatively on both linear growth and spore germination. Complete inhibition of linear growth and spore germination were observed with lemongrass oil and thyme oil vapors at concentration of 100.0µl/L of each. Meanwhile, the same effect was recorded at concentration of 0.50 ml/L. *In vivo* pre-inoculated strawberry fruits were submersed in a concentration of 2.0 ml/L of essential oil emulsion lemongrass and thyme oils, a method that ensured complete penetration of the oil into treated fruits resulted in about 90.0% reduction in disease incidence. This was obsereved for the two phases used of essential oil and peracetic acid. A similar feature of the efficacy of lemongrass oil, thyme oils and preacetic acid were used to inhibit gray mould incidence at all concentration. These results concluded that one tenth of each chemicals tested was enough to gain such results if used as vapor phase. These results suggested that lemongrass oil, thyme oils and preacetic acid triggered different mechanism for inhibiting gray mould disease depending on their applied phases.

**Key words:** *Botrytis cinerea,* Gray mold, Fumigation, Lemongrass, Peracetic acid, Strawberry, Thyme

## Introduction

Strawberry (*Fragaria* × *ananassa*) is among the most perishable fruits and is vulnerable to physical injuries and fungal invasion. Gray mold caused by *Botrytis cinerea* Pers. ex. Fr. is the most economically important postharvest disease of strawberry fruits that causes losses before or after harvest (Elad *et al*., 2004; Williamson *et al.,* 2007; Zhang *et al*., 2007). Strawberries deteriorate

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during storage as a result of decay along with physical senescence and dehydration. *Botrytis cinerea* is the most frequently reported as decay fungus responsible for microbial deterioration of strawberries (Mass, 1981). *B. cinerea* can cause postharvest decay in strawberry from surface-borne inoculum that infects the fruit through injuries or micro-wounds located on any part of the skin, but decay originating from blossom latent infections is frequently more important. Typically, the pathogen infects the flowers or the crown of young fruits in the field, remains latent, and after harvest develops from the crown to the rest of the mature fruit causing an apparent brown discoloration of the skin (Droby and Lichter, 2004). Additionally, *B. cinerea* is able to infect stored strawberries by mycelial spread from infected fruit to adjacent healthy fruit, causing 'nests' of decay under favored conditions. Postharvest diseases caused by pathogenic fungi resulting in major losses of fruits and vegetables, and synthetic chemical fungicides are the primary means to application at present (Spadaro and Gullino, 2004). However, synthetic chemical fungicides are potentially harmful on human health and the emergence of pathogens which are resistant to these chemicals (Holmes and Eckert, 1999). Moreover, public concern over the indiscriminate use of synthetic fungicides has been growing. Thus, it is significant to develop new alternatives for disease control measures (Tian, 2006).

Essential oils are also considered a promising alternative with many having antifungal properties. However, very high concentration is needed when applied to real food systems (Hammer *et al*., 2003; Ahmet *et al*., 2005). Application of essential oil is a very attractive method for controlling postharvest diseases. Essential oils and their components are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional use (Ormancey *et al*., 2001). In this regard, lemongrass (*Cymbopogon citratus* L.) oil was reported to be antifungal activity against several plant pathogens. Fungal spore production, spore germination and germ tube length of *C. coccodes, B. cinerea, C. herbarium* and *R. stolonifer* was inhibited with lemongrass oil treatments (Tzortzakis and Economakis, 2007). Moreover, using lemongrass essential oils by spraying or dipping fruits for controlling postharvest diseases of several fruits has been reported (Somda, *et al.,* 2007; Tzortzakis and Economakis, 2007). Also, thymol is an essential oil component from thyme (*Thymus capitates* L.) and has been used as medicinal drug, food preventative, and beverage ingredient (Mansour *et al.,* 1986) as well as plant diseases of several fruits and vegetables ( El- Sherbieny, *et al.,* 2002, Plaza, *et al.*, 2004 Angelini, *et al.,* 2006 Feng and Zheng, 2007 and Klaric, *et al.,* (2007). Peracetic acid is another material that has greater stability and faster

biocidal properties and could be used as postharvest disinfectant (Mari *et al*., 1999). Peracetic acid (PAA) was early used as patent in 1950 to treat fruits and vegetables to reduce spoilage from bacteria and fungi destined for processing. It has been used to disinfect with water washing and used to handle fresh produce. It is used to treat bulbs and in seed treatment to inactivate fungal or other types of plant disease (Wright *et al*., 2000; Hanks and Linfield, 1999 and Hei, 2000). Also, Kyanko *et al*. (2010) concluded that the peracetic acid is a nonpolluting alternative treatment for post-harvest rotting control of fruits and vegetables.

The purpose of the present study was to evaluate the effect of Lemongrass, Thyme essential oils and peracetic acid either in their vapor or liquid phases on the *in vitro* growth and spore germination of fungus *B. cinarea*. Moreover, their effects against the gray mould incidence of strawberry fruits were also tested under *in vivo* conditions.

## Materials and methods

### *Source of pathogenic fungus and strawberry fruits*

A pathogenic isolate of *Botrytis cinerea* Pers. ex. Fr., the causal agent of gray mold disease of strawberry fruits obtained kindly from Culture Collection Unit, Department of Plant Pathology, National Research Centre, Giza, Egypt. Meanwhile, Fresh harvested healthy of strawberry fruits cv. Sweet Sharly were purchased from El-Ebour commercial principal market at Cairo, Egypt.

### *Source of plant essential oils*

Essential oils of lemongrass oil (*Cymbopogon citrates*) and thyme oil (*Thymus capitatus*) were purchased from International Flavors and Plant oils Inc., Giza, Egypt. These essential oils were stored in dark bottles at 4ºC for further studies.

### *Fumigation chamber*

Fumigation was carried out in specially designed fumigation chamber 270 L in volume which supplemented with fan on the top, to have closed circulated air current, and three shelves (Morsy *et al*., 1999).

### *Preparation of spore suspension*

Spores of 10-day old cultures of *B. cinerea* were harvested in sterilized water containing 0.01 % Tween 80, then adjusted through sterilized water serial

dilutions to reach concentration of $10^6$ spore/ml with the aid of haemacytometer slide.

***In-vitro tests***

The essential oils, *i.e*. lemongrass (LO) and thyme (TO) oils as well as peacetic acid (PAA) were tested for their inhibiting effect against *B. cinerea* under in-vitro conditions. Four concentrations of LO, TO and PAA at their vapor phases of 0.0, 25.0, 50.0 and 100.0 µl/l were tested against linear growth of the fungal pathogen. The fumigation chamber was used for this purpose (Morsy *et al*., 1999). A volume of 100 mL of tested chemical was poured into uncovered Petri-dishes (18 cm diameter) and placed under the chamber's fan. Disks of 6- mm-diameter of 10 days old cultures of *B. cinerea* were placed into uncovered Petri-dishes which distributed into chamber's shelves and exposed individually to fumigation released from the different tested chemicals for 30 min. in fumigation chamber, then transferred to the centre of another plates containing potato dextrose agar (PDA). Fungus linear growth was measured for all treatments when the control plates (untreated fungal disks) reached full growth. Twenty five replicates were used for each particular treatment. The average diameter of fungal growth at all treatments was calculated. The efficacy of LO, TO and PAA at the same concentrations for inhibiting spore germination of *B. cinerea* under in-vitro conditions was evaluated. One drop (0.1 mL) of spore suspension ($10^6$ spore/ml) of *B. cinerea* was placed onto slide which lied into uncovered Perti-dishes. All uncovered Petri-dishes were exposed individually to fumigation released from the different tested chemicals for 30 min. in fumigation chamber. The treated slides were transferred onto moistened filter paper placed into Petri-dishes. All Petri-dishes were covered and incubated for 24h at 25$^o$C. Spore germination was determined microscopically as percentages by counting the field containing100 spores five times in each drop (Sholberg and Gaunce, 1995).

Evaluating the efficacy of LO, TO and PAA in their liquid phase against linear growth and spores germination of *B. Cinerea* was also carried out *in vitro* conditions. Four concentrations of LO, TO and PAA solutions of 0.0, 0.25, 0.50 and 1.0 ml/L were tested. Essential oils and PAA compounds solutions were added to conical flasks containing autoclaved PDA medium before solidifying to obtain the proposed concentrations, then mixed gently and dispensed in sterilized Petri dishes (9 cm–diameter). Petri dishes were individually inoculated at the center with equal disks (6-mm diameter of 10 days old culture of *B. cinerea*. Transferred Petri dishes were incubated at 25±2°C. Fungal linear growth was measured when the control plates reached full growth, and then the average diameter was calculated. Twenty five replicates were used for each

particular treatment. As for spore germination test, the fungal spore suspension was prepared individually into each tested concentration of tested chemicals. Then, the same consequences were followed as metioned above.

### *In-vivo tests*

Evaluation of the essential oils, LO and TO as well as peacetic acid (PAA) treatments against gray mould incidence of strawberry fruits was carried out *in-vivo* conditions. Fresh strawberry fruits apparently healthy and free from physical damage were used. Fruits were inoculated by spraying with spore suspension ($1x10^6$ spores/mL) of *B. cinerea*, then air dried at room temperature (23-25C°). The LO, TO and PAA at four concentrations of 0.0, 50, 100 and 200 µl/L were tested as vapor phase as stated above. Strawberry fruits were placed into pierced foam plates, to allow vapor circulation, which distributed into chamber's shelves and exposed individually to fumigation released from the different tested chemicals for 30 min. in fumigation chamber, then transferred to egg carton trays (30x30cm with 30 holes each). As for evaluation of LO, TO and PAA in their liquid phase, concentrations of 0.0, 0.50, 1.0 and 2.0 ml/L were tested. Inoculated strawberry fruits were dipped in each prepared concentration tested for one min., then air dried at room temperatures and transferred to egg carton trays. Another set of un-treated fruits was served as control treatment. Five trays were used for each particular treatment. All trays were stored in cold room at 10-15°C for 14 days, with daily examination. Decayed fruits were counted and the percentage of disease incidence was recorded at the last of storage period.

### *Statistical analysis*

Tukey test for multiple comparison among means was utilized (Neler *et al.,* 1985).

## Results and discussion

### *In-vitro test*

The efficacy of the essential oils, lemongrass (LO), thyme (TO) oils and peracetic acid (PAA) on linear growth and spores germination of *B. cinerea* are presented in Tables 1 and 2. It is indicated that all vapor treatments significantly reduced the linear growth and spore germination of *B. cinerea*. It was also observed that rising concentration of tested chemicals reflected negatively on both linear growth and spores germination. Complete inhibition

of linear growth and spore germination was observed with LO and TO vapors at concentration of 100.0 µl/L of each. Treated fungus with LO and TO vapors at concentration of 50.0 µl/L of each, resulted in reduction in the linear growth as 75.5 and 80.0% and spore germination as 82.1 and 84.2%, respectively. Meanwhile, other treatments showed lesser effect.

Concerning the efficacy of LO, TO and PAA in their liquid phases on linear growth and spores germination of *B. cinerea,* the obtained results are presented in Table 2. Both fungal growth and germinated spores increased in ascending order as the concentrations of tested chemicals are reduced to reach its maximum at concentration of 0.25 ml/L. Complete reduction in mycelial fungal growth was observed at concentration of 0.50 ml/L of both LO and TO essential oils, whereas concentration of PAA caused only 54.4% reduction in fungal growth at the same concentration.

The response of spore germination against tested chemicals showed similar recorded curve for fungal growth (Tables 1 and 2) following the same observation when treated with LO, TO and PAA either in their vapor or liquid phases.

**Table 1.** Effect of fumigation with essential oils and peracetic acid on the linear growth and spores germination of *B. cinerea in-vitro*

| Treatment | Concentration µl/L | Linear growth (mm) | Reduction % | Spore germination | Reduction % |
|---|---|---|---|---|---|
| Lemongrass oil (LO) | 25.0 | 35.0 f | 61.1 | 32.0 e | 66.3 |
| | 50.0 | 22.0 g | 75.5 | 17.0 f | 82.1 |
| | 100.0 | 00.0 h | 100 | 00.0 g | 100 |
| Thyme oil (TO) | 25.0 | 28.0 f | 68.8 | 25.0 e | 73.6 |
| | 50.0 | 18.0 g | 80.0 | 15.0 f | 84.2 |
| | 100.0 | 00.0 h | 100 | 00.0 g | 100 |
| Peracetic acid (PAA) | 25.0 | 75.0 b | 16.6 | 69.0 b | 27.3 |
| | 50.0 | 61.0 d | 32.2 | 54.0 d | 43.1 |
| | 100.0 | 40.0 e | 55.5 | 32.0 e | 66.3 |
| Control | 0.0 | 90.0 a | --- | 95.0 a | --- |

Figures with the same letters are not significant (P≤0.05).

**Table 2.** Effect of essential oils and peracetic acid as liquid phase on the linear growth and sporesgermination of *B. cinerea in-vitro*

| Treatment | Concentration ml/L | Linear growth (mm) | Reduction % | Spore germination | Reduction % |
|---|---|---|---|---|---|
| Lemongrass oil (LO) | 0.25 | 32.0 d | 64.4 | 25.0 c | 73.6 |
| | 0.50 | 00.0 e | 100 | 00.0 d | 100 |
| | 1.00 | 00.0 e | 100 | 00.0 d | 100 |
| Thyme oil (TO) | 0.25 | 25.0 d | 72.2 | 22.0 c | 76.8 |
| | 0.50 | 00.0 e | 100 | 00.0 d | 100 |
| | 1.00 | 00.0 e | 100 | 00.0 d | 100 |
| Peracetic acid (PAA) | 0.25 | 52.0 b | 42.2 | 44.0 b | 53.6 |
| | 0.50 | 41.0 c | 54.4 | 31.0 c | 67.3 |
| | 1.00 | 30.0 d | 66.6 | 25.0 c | 73.6 |
| Control | 0.0 | 90.0 a | --- | 95.0 a | --- |

Figures with the same letters are not significant (P≤0.05).

Result showed that the efficacy of LO, TO and PAA in their vapor phases gave a higher effect on linear growth and spores germination of *B. cinerea* comparing to their effect in a liquid phase (Tables 1 and 2). This observation could be attributed to the diffusible absolute chemicals concentration into the fungal growth disks before cultured onto media as well as spores exposed to the same conditions comparing with their direct effect against the fungal propagules when used in water solution. Referring to the tested chemicals in the present study, it should be taken into consideration that concentrations of LO, TO and PAA as liquid phase represented ten folds that used as vapor phase. The effect of LO, TO and PAA on spore germination and mycelial growth were compared as seen in Tables 1 and 2. It was observed that the three potential antimicrobial displayed different efficacy to *B. cinerea* at different developmental stages. At the mycelial growth stage, both chitosan and oligochitosan appeared higher effective on the fungal growth than that at the spore germination stage (Tables 1 and 2) that most likely due to a stronger drug resistance of fungal spores. Moreover, the mycelial growth of *B. cinerea* was more sensitive to LO and TO than that of PAA. This observation was true in both cases of chemicals tested. In this regard, vapors of thyme, oregano and lemongrass, and their respective major components showed completely growth inhibition of *Botrytis cinerea* and *Alternaria arborescens* as reported by Plotto *et al*. (2003). Also, Arrebola *et al*. (2009) recorded that Thyme (TO) and lemongrass oils (LO) showed over 50% and 25% inhibition of radial mycelial growth respectively. Jaspers *et al*. (2001) reported that *B. cinerea* sporulation on artificially induced necrotic leaf lesion was significantly reduced by thyme

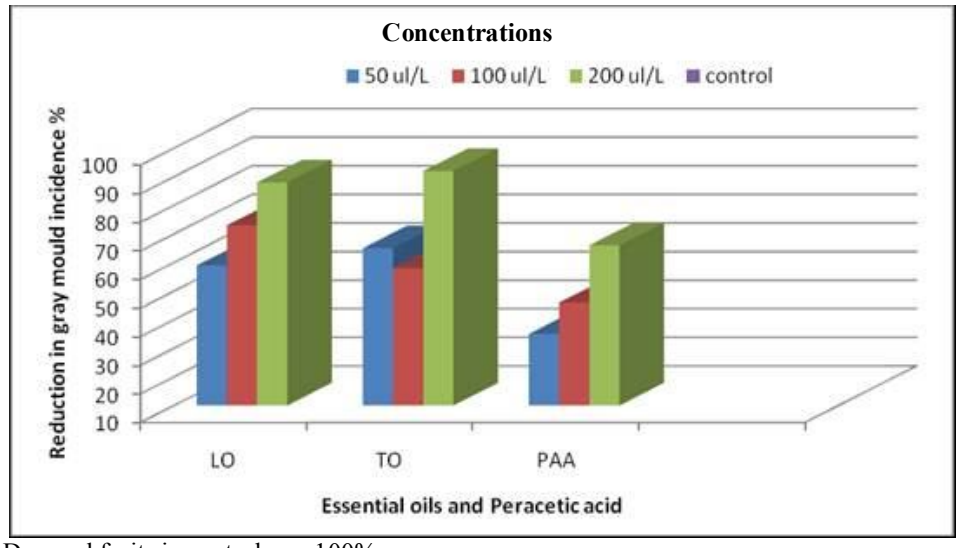
oil at concentration of 0.33%. In this respect, Tzortzakis and Economakis (2007) reported that lemongrass oil expressed antifungal activity against *Colletotrichum coccodes, B. cinerea, Cladosporium herbarium, Rhizopus stolonifer* and *A. nige*r *in vitro*. They also reported that lemongrass oil at 25 ppm could inhibit spore production and at 500 ppm, the highest oil concentration employed, fungal sporulation was completely inhibited. Lemongrass oil could reduce spore germination and germ tube length of *C. coccodes, B. cinerea, C. herbarium* and *R. stolonifer*.

***In-vivo tests***

Essential (volatile or ethereal) oils are the concentrated oils of plants that contain volatile aroma compounds. These oils naturally contain bioactive compounds that can effectively manage growth and spores germination of *B. cinerea* (Tables 1 and 2). Many essential oils were shown to be effective for inhibiting *B. cinerea in vitro* (Jaspers *et al*., 2001; Bouchra *et al*., 2003; Daferera *et al.,* 2003). The use of lemongrass oil (LO) to control *B. cinerea* was reported by Tzortzakis and Economakis (2007). The active component of thyme oil (TO), thymol, controlled *B. cinerea* and retained the overall quality of table grapes in modified atmosphere packaging (MAP) was indicated by Valverde *et al*. (2005). Essential oils (EOs) and their components are gaining increasing interest due to their volatility, relatively safe status, wide acceptance by consumers, eco-friendly and biodegradable properties (Tzortzakis, 2007). Application of EOs is an attractive method to control postharvest diseases in postharvest systems due to their bioactivity in the vapour phase and the limitation of aqueous sanitation for many commodities, make them useful as possible fumigants.

In present study, result indicated that all treatments significantly reduced the gray mold incidence of strawberry fruits as illustrated in Figures 1 and 2. The most effective treatments were LO and TO vapors at concentration 200 µl/l for each, which reduced the disease incidence by 88.0 and 92.0% respectively meanwhile PAA was lower effect (66.0%) at the same concentration (Fig. 1). The other concentrations showed descending lesser effect to reach its minimum at 50µl/L. Similar results were obtained with dipping fruits in solutions of tested treatments (Fig. 2). Using lemongrass and thyme essential oils by spraying or dipping fruits for controlling postharvest diseases of several fruits were reported by several investigators (Bhaskara *et al*., 1997; Somda *et al.,* 2007; Tzortzakis and Economakis, 2007). In the present work, pre-inoculated strawberry fruits were submersed in a concentration of 2.0 ml/L essential oil emulsion (LG and TO), a method that ensured complete penetration of the oil into treated fruits resulted in about 90.0% reduction in disease incidence. This

observation was true for the two phases used of essential oil and peracetic acid. A similar feature of the efficacy of LG, TO and PAA against gray mould incidence at all concentration used. These results lead to conclude that one tenth of each tested chemical is enough to gain such results, if used as vapor phase. This conclusion was also reached by Plotto *et al*., (2003) who reported that vapors of thyme, oregano and lemongrass, and their respective major components showed completely growth inhibition of *B. cinerea* and *Alternaria arborescens*. Moreover, Tripathi and Dubey (2004) reported that some of the essential oils were reported to protect stored commodities from deterioration. They stated that bioactivity in the vapour phase of essential oils that was recognized as a characteristic of attractive as possible fumigants for stored product protection. Also, Emulsions of thyme and oregano oils at 5,000 ppm and 10,000 ppm as dip treatments reduced disease development in tomatoes inoculated with *B. cinerea* and *A. arborescens,* respectively (Plotto *et al*., 2003).

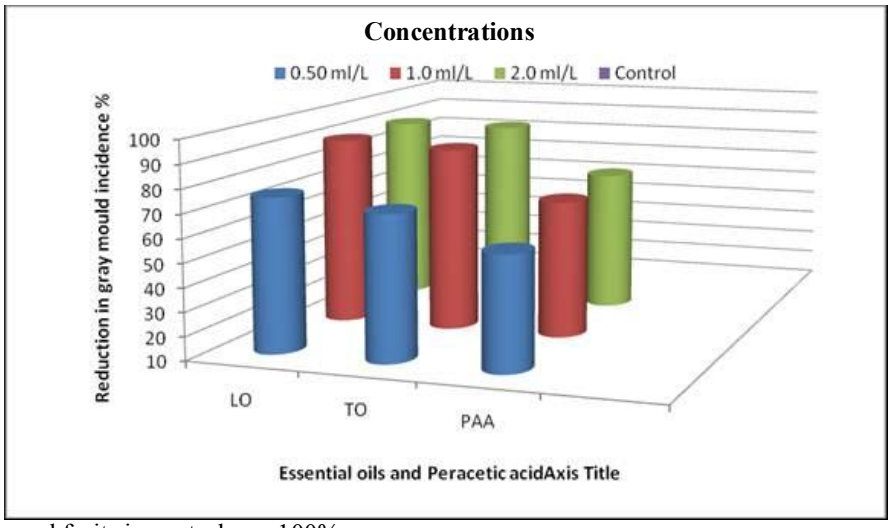



* Decayed fruits in control was 100%

**Fig. 1.** Reduction in gray mould incidence in response to fumigated Strawberry fruits with LG and TO essential oils and PAA on gray mold incidence under *in-vivo* conditions

Thymol is an essential oil component from thyme and has been used as medicinal drug, food preventative, and beverage ingredient as well as plant diseases of several fruits and vegetables (Mansour *et al.,* 1986). In this respect, Liu *et al.* (2002) found that thymol was more effective for controlling brown rot symptoms on apricots, and fumigation of plumus with relatively low concentrations can greatly reduce postharvest decay without causing any

phytotoxicity. Moreover, Plaza, *et al.*, (2004) evaluated the potential of using essential oils instead of synthetic fungicides to control *P. digitatum* and *P. italicum* on citrus fruits by determine the antifungal activity of 20 essential oils on colony growth of *P. digitatum* and *P. italicum in vitro* and to evaluate their efficacy *in vivo* by simulating fungicide application in the packing line or incorporating them into packaging of oranges. Thyme oil completely inhibited *P. digitatum* and *P. italicum* growth either when added into the medium at 1000 1ug/L or by their volatiles. *In vivo* thyme and cinnamon essential oils significantly reduced the incidence of *P. digitatum* and *P.italicum* after 12 days at 20$^{o}$C. Furthermore, Klaric *et al.*, (2007) reported that antifungal activities of the thyme essential oil, which contains p-cymene (36.5%), thymol (33.0%) and cineole (11.3%) as a main components, and pure thymol were determined by the dilution method and exposure to vaporous phase of the oil. Thymol exhibited approximately three times stronger inhibition than essential oil of thyme. The vaporous phase of the thyme essential oil strongly suppressed the linear growth and sporulation of *Pencillium* sp. *Alternaria* sp., *Absidia* sp., *Mucor* sp., *Cladosporium* sp. and *Rhizopus* sp. Using thyme oils for controlling postharvest disease of several fruits are also reported (Plaza, *et al.*, 2004; Angelini, *et al.,*2006; Feng and Zheng, 2007 and Klaric, *et al.,* 2007).




* Decayed fruits in control was 100%

**Fig. 2.** Reduction in gray mould incidence in response to submersed strawberry fruits in LG and TO essential oils and PAA solutions on gray mold incidence under *in-vivo* condition

In the present study, peracetic acid was significantly reduced the linear growth and spore germination of *B. cinerea*, and reduced the gray mold disease of strawberry fruits. Peracetic acid as treatment for fruits and vegetables to

reduced spoilage from bacteria and fungi destined for processing (Wright *et al.,* 2000, Hanks and Linfield, 1999 and Hei, 2000). In this respect, Block (1992) reported that peracetic acid as the peroxide of acetic acid, acts as a disinfectant that desirable properties of hydrogen peroxide, *i.e*. broad spectrum activity against microorganisms, lack harmful decomposition products and infinite water solubility. Peracetic acid also has excellent sporocidal activities (Alasri *et al.,* 1993).

This work highlighted the potential for using essential oils for postharvest disease control of fresh fruit and vegetables. Essential oils (LO and TO) as well as peracetic acid (PAA) which have been registered as Food Additives are much easier to register for postharvest use than new synthetic pesticides. Application of these oils via the vapor phase made their use more cost effective than dipping. The the optimum concentration of oil for maximum control of the pathogens with acceptable levels of tainting of the product are being investigated.

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