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Efficacy of essential oil vapour phase against post-harvest fungal pathogen *Penicillium digitatum* isolated from *Citrus reticulata*

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The present study envisages the efficacy of essential oils (EOs) namely basil oil, ginger oil and lavender oil for management of decay of *Citrus reticulata* with an objective to impede major post-harvest diseases and to prolong the storage life without altering any fruit quality parameter. The research is being strategized in view of getting alternative post-harvest solution for organic value chain. An antifungal activity of essential oils against isolated fungus *Penicillium digitatum* was assessed by vapour phase activity. The results indicated that Ginger oil at the concentration of 80µg ml⁻¹ showed strong mycelia growth inhibition followed by basil oil and lavender oil at concentration of 160µg ml⁻¹, 480µg ml⁻¹ respectively. An *in vivo* trial on *Citrus reticulata* also supported these results. At all the stages of storage, green mold decay was found less in EOs treated fruits than the untreated one and hence increased their storage life significantly.

Key words: *Citrus reticulata*, *Penicillium digitatum*, essential oils, vapour phase activity, ginger oil

INTRODUCTION

India is known to be the major citrus growing and exporting country and a homeland for several citrus species. Mandarin (*Citrus reticulata* Blanco) is one of the important horticulture crops grown mainly in Assam, Tamil Nadu, Karnataka, Madhya Pradesh, Maharashtra, Mizoram, Nagaland, Rajasthan, West Bengal and Sikkim. In Sikkim, it is organically grown in an area of 9457 ha with an average production of 16,850 metric ton (MT) and productivity of 3,312 Kg/ha (Anonymous 2014). Post-harvest diseases following mishandling of the harvested fruits, contribute about 30-40% of fruit loss worldwide and which is much higher in developing countries (Bhattarai *et al.* 2013). Mycotoxin producing fungus, especially belonging to *Penicillium*, *Aspergillus* and *Alternaria* species, are responsible for food spoilage. The wound pathogens, green mold (*P. digitatum*) and blue mold (*P. italicum*) account for most of the decay of citrus fruit worldwide. In many countries including India, these diseases are primarily controlled by the extensive use of fungicides, such as orthophenylphenate,

imazalil, and thiabendazole as pre- or post-harvest treatments (Moorman and Lease, 1992). Increasing concerns about chemical usage and their residual impact in food and the environment, there is a renewed interest in organic approaches to control different postharvest diseases (Vaughn *et al.* 1993).

There is, therefore still a need for new and effective natural antimicrobial compounds to reduce and inhibit the pathogenic fungi. In nature, essential oils play an important role in the defensive mechanisms of plants. Most of the reported essential oils have antibacterial, antiviral, antifungal and insecticidal properties (Bakkali *et al.* 2008). Thus, the use of biologically based compounds, such as essential oils obtained from medicinal plants, can serve as a feasible approach for reducing post-harvest diseases in harvested fruits and vegetables. The use of such alternative method for controlling post-harvest diseases will help to combat fungicide resistant strains of pathogens and will avoid ever increasing pesticides residues in the environment and commodities, thus, minimizing the effects on non-target microorganisms.

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The present investigations envisage studying the effects of essential oil for controlling the post-harvest disease of organically grown *Citrus reticulata* Blanco. The objective of this study was to evaluate the in-vitro and in-vivo antifungal activity of essential oils in vapour phase of Ginger (*Zingiber officinale*), Basil (*Ocimum basilicum*) and Lavender (*Lavandula stoechas* L. subsp. *stoechas* L.). The vapour phase activity is preferred over suspending oil in aqueous medium because the lipophilic molecules forms micelles in the aqueous medium and thus suppress the attachment of the essential oils to the organism (Inouye *et al.* 2003).

MATERIALS AND METHODS

Plant materials and extraction of essential oils

Plants materials (Ginger rhizome and Basil leaves) were collected from West Sikkim district, Sikkim, India. Oil extraction was done using a Clevenger-type apparatus where the dried rhizome of ginger and dried basil leaves were subjected to hydro distillation. The plant material and double distilled water was mixed in the ratio of 1: 10 and subject to hydro distillation for 4 hours. The essential oil was dried over anhydrous Na₂SO₄ and preserved in a sealed vial at 4 p C. However, Lavender oil was purchased from the local market.

Fruit sample

Infected orange fruits (*Citrus reticulata*) were collected from the identified farmer's field of East Sikkim during the harvesting period.

Isolation of test pathogen and preparation of spore suspension

The infected fruit was surface sterilized by 2% sodium hypochlorite for 3 minutes followed by rinsing with sterilized distilled water. An infected portion of fruit was sliced out with sterile blade, and placed in sterilized potato dextrose agar amended with streptomycin (30µg ml⁻¹) under aseptic conditions. It was then incubated at 28°C for 7 days. The culture was obtained by repeated sub-culturing. The pure fungal culture was maintained in sterile PDA slants at room temperature and refrigerated condition (4°C) throughout the study period. The spore suspension was prepared by gently removing the spores from the sporulating edges of 7-8 days old culture with

a bacteriological loop and suspending them in sterile distilled water.

Morphological Identification

The morphological characteristic of fungal mycelium viz. colony growth pattern, conidial morphology and pigmentation was recorded. Fungal identification isolated from the diseased *Citrus sinensis* fruits was done by slide culture techniques (Oyeleke and Manga, 2008) and confirmed with the help of Mycological Atlas of Robert and Ellen (1988)

Antifungal activity of essential oils on mycelial growth

The antifungal activities of volatile phase of essential oils on mycelial growth of *Penicillium digitatum* was assessed as described by Soylu *et al.*(2007). For determination of volatile phase effect, glass Petri dishes (90 x 20 mm, which offer 80 ml air spaces after addition of 20 ml agar media) were used.

The mycelial disc of 7 days old *Penicillium digitatum* culture was cut with the help of sterile cork borer (6mm) and placed at the centre of Potato dextrose media. Following inoculation in media, the sterile filter papers disc (6mm diameter, Whatman no.1) impregnated with different concentrations of essential oils (0.8, 1.6, 3.2, 6.4, 9.6, 19.2, 25.6, 32.0, 38.4, 44.8 and 56.0 mg ml⁻¹) were placed in the center of the inverted lid of Petri dishes to obtain final concentrations (10, 20, 40, 80, 120, 160, 240, 320, 400, 480 and 560 µg ml⁻¹ air). The Petri dishes were sealed immediately with parafilm to prevent loss of essential oil vapors and incubated at 20-22°C. The mean radial mycelial growth of the pathogen was determined by measuring the diameter of the colony in two directions at right angles to each other after 7 days of incubation period. The growth was compared to the control plate in which the fungus covered the plate 7 days after inoculation. For each concentration, three replicates were prepared. The mean growth values were obtained and then converted into the inhibition percentage of mycelial growth in relation to the control treatment (without essential oil) by using the formula: Mycellial growth inhibition = (dc-dt)/dc x 100 (where dc and dt represent mycelial growth diameter in control and treated Petri plates, respectively).

Quality indexing after application of essential oils in in-vivo condition

The antifungal activity was assessed *in vivo*, the citrus fruits were dipped in the solution of 3% Sodium hypochlorite for 2 minutes, properly rinsed with distilled water, and air-dried before treatment. Air-dried fruits were inoculated with the spore suspension (10^6 spore ml^{-1}) of test pathogens and allowed to dry. The inoculated fruits were treated with different concentration of selected essential oil and commercial fungicide. Treated fruits were stored at room temperature to measure the disease incidence. The treatment was designed based on CRD (Completely Randomized Design). Treatment used for the tests were as follows: T0 = Control, T1 = Basil Oil (500 ppm), T2 = Basil Oil (750 ppm), T3 = Basil Oil (1000 ppm), T4 = Ginger Oil (500 ppm), T5 = Ginger Oil (750 ppm), T6 = Ginger Oil (1000 ppm), T7 = Lavender Oil (500 ppm), T8 = Lavender Oil (750 ppm), T9 = Lavender Oil (1000 ppm) and T10 = Fungicide (Bordeaux)

The infection incidence and severity of disease

The degree of infection on fruit was rated using a scale of 1 to 9 after 7 days of treatment, where 1= no infection; 2= trace infection lower than 10%, 3= infection between 10- 20%, 4= infection between 20- 30%, 5= infection between 30- 40%, 6= infection between 40- 50%, 7= infection between 50- 65%, 8= infection between 65- 80% and 9 = infection more than 80%.

Weight loss (%): This character was determined by weighing fruit in each stage and calculated as weight loss percentage.

Spoilage percentage: Spoilage percentage was calculated by dividing the number of decayed fruits by the total number of fruit as per the following equation:

Biochemical parameter

Total soluble solids (TSS): Total soluble solids (TSS) in fruit juice was determined by using a digital refractometer.

Total acids: Total acidity in fruit juice was determined by titrating filtered fruit juice against 0.1 NaOH with phenolphthalein as an indicator and calculated as gram of citric acid per 100ml fruit

juice by the method as described by the association of analytical chemistry (AOAC 2016).

Statistical analysis: All data obtained throughout the experiment was tabulated and statistically analyzed and significance of the difference among treatment mean was calculated using T-Test. Analysis of variance was performed at the significance level of $P < 0.05$.

RESULTS AND DISCUSSION

Post-harvest decay has been the growing concern in food industries. Application of essential oil has been considered as an alternative for management of postharvest pathogen and in turn the desirable means for eliminating synthetic fungicides. Thereby, essential oil-amended coating of fruits to maintain the fruit quality may also comply with the consumer preferences and organic certification.

Isolation and identification of fungus

Fungal colonies on potato dextrose agar was grown to give olive green appearance of spores surrounded by white mycelia. On microscopic examination under 40X objective lens, a chain of single cell conidia with cylindrical phialides was observed (a characteristic feature of green mold fungus). Based on cultural and morphological characteristics and confirmation with reference to Mycological Atlas of Robert and Ellen (1988) the fungus was identified as *Penicillium digitatum*.

Mycelial Inhibition

All the essential oils showed moderate to high fungistatic and fungicidal effects on *P. digitatum*. Study of an effective concentration of essential oil to inhibit the mycelial growth showed a dose-dependent inhibition pattern (Table 1). Of the three oils, ginger oil exhibited the strongest antifungal activity against *P. digitatum*. In a treatment with ginger oil, complete mycelial inhibition was observed at 80 $\mu\text{g ml}^{-1}$ air. The inhibition of fungal mycelia was, however, totally inhibited at a higher concentration of *O. basilicum* (160 $\mu\text{g ml}^{-1}$ air) and *L. stoechas* (480 $\mu\text{g ml}^{-1}$ air) oil. As the higher concentration of basil and lavender oil is required to inhibit fungi, they were determined as fungistatic while inhibition of fungal growth at the minimum concentration of ginger oil was determined as

Table 1 : Effects of different concentration of volatile phases of essential oils on the mycelial inhibition

Essential oils and mycelial inhibition (%)			
Concentration ($\mu\text{g ml}^{-1}$ air)	<i>Z. officinale</i>	<i>O. basilicum</i>	<i>L. stoechas</i>
0	0 ^a	0 ^a	0 ^a
10	53.56 ^d	26.35 ^b	16.59 ^b
20	78.37 ^f	40.28 ^c	22.37 ^b
40	86.68 ^g	67.39 ^e	28.67 ^b
80	100.00 ⁺⁺⁺	77.54 ^f	37.85 ^c
120	-	88.27 ^g	46.63 ^c
160	-	100.00 ⁺⁺	62.37 ^e
240	-	-	78.64 ^f
320	-	-	84.43 ^g
400	-	-	93.22 ^h
480	-	-	100.00 ⁺⁺
560	-	-	-

●The mean radial mycelial growth of *P. digitatum* was determined at 7 days after the inoculation. Each observation is based on 3 replicates. Mean values followed by different letters within the column are significantly different according to t-test ($P < 0.05$). Experiments were repeated twice. + and ++ symbols indicate that antifungal effects at shown concentrations are fungistatic and fungicidal respectively

Table 2: Average weights of the fruit at different storage time (Mean \pm SE)

Treatment	Day 0	Day 5	Day 10
T0	58.91 \pm 2.39	58.24 \pm 2.54	53.06 \pm 2.12
T1	60.45 \pm 2.21	59.90 \pm 2.62	57.58 \pm 2.11
T2	61.76 \pm 2.23	59.21 \pm 2.38	55.70 \pm 2.22
T3	63.38 \pm 2.15	61.75 \pm 2.15	55.54 \pm 2.36
T4	62.02 \pm 1.95	60.35 \pm 2.24	58.09 \pm 2.64
T5	62.90 \pm 1.75	60.23 \pm 2.32	59.50 \pm 2.43
T6	60.47 \pm 2.64	58.57 \pm 2.32	57.85 \pm 2.16
T7	62.62 \pm 2.75	62.50 \pm 2.45	58.98 \pm 2.46
T8	59.26 \pm 1.76	58.32 \pm 2.72	56.46 \pm 2.34
T9	63.52 \pm 2.43	61.08 \pm 2.41	57.66 \pm 2.38
T10	72.71 \pm 2.62	70.45 \pm 2.22	69.82 \pm 2.67

fungicidal. Lavender, Basil, and Ginger oil have been previously reported to have antimicrobial activity against various pathogenic fungi and bacteria (Inouye *et al.* 1998; Singh *et al.* 2012), showing a promising potential to improve the shelf life of perishable foods.

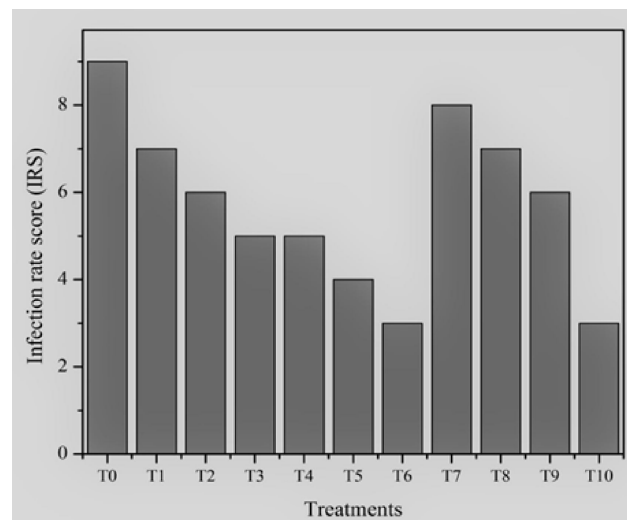
Disease Incidence

Ginger oil at the concentration of 100ppm (T6) was able to reduce disease by more than 70% as compared to control, where more than 90% fruits were severely decayed due to green mold. Interestingly the result is at par with the synthetic fungicide (T10). In contrast, Lavender oil did not show high inhibition of green mold on oranges after

7 days of wound inoculation Fig.1a. This result shows a promising indication towards the organic application of such essential oil in lieu of synthetic fungicides which have residual effects. Reduction in disease incidence due to the application of essential oils can be observed in several reports (Pradhanang *et al.* 2003; Kouassi *et al.* 2012; Jhalegar *et al.* 2015).

Average weight loss of the fruit

Fresh weight of the fruit is one of the basic biological parameters to determine the quality, which usually reduces with the increase in decay. After essential oil treatment, a gradual and steady reduction in weight was observed in a dose-dependent manner in comparison with control. Fresh weight of the fruits on day 0 was found in the range of 58.91 to 72.71g/fruit. After 5 days of treatment, insignificant changes in weight was observed. As the treatment period increases to ten

**Fig. 1a :** Quality index after application of essential oils : Disease incidence

days, an increase in reduction of fresh weight was observed. Maximum fresh weight after ten days was observed in the treatment T10 (69.82g \pm 2.67) and found statistically different with treatment T5 (59.50g \pm 2.43), T4 (58.09 \pm 2.64), T6 (57.85 \pm 2.16), T9 (57.66g \pm 2.38), T3 (55.54g \pm 2.36), T4 (58.09g \pm 2.64), T7 (58.98g \pm 2.46) and minimum fresh weight was observed under treatment T0 (53.06g \pm 2.12) (Table. 2). Calculation of weight loss percentage demonstrated the efficacy of the essential oils in storage and preservation. The ginger oil was found to be most effective oil against the green mold infection in storage condition. The

percentage of weight loss in T4 was negligible with a loss of 2.76% at fifth days of storage which merely increased to only 6.77% after ten days of storage. As the concentration of ginger oil was gradually increased in T5 and T6 treatment, less weight reduction was observed. In tenth days of storage, in T5 5.71% and in T6 4.52% of fruit weight loss was observed Fig. 1b. The result is corroborated with the studies of Al-Samarrai *et al.*(2013) and Someshwar *et al.* (2016).

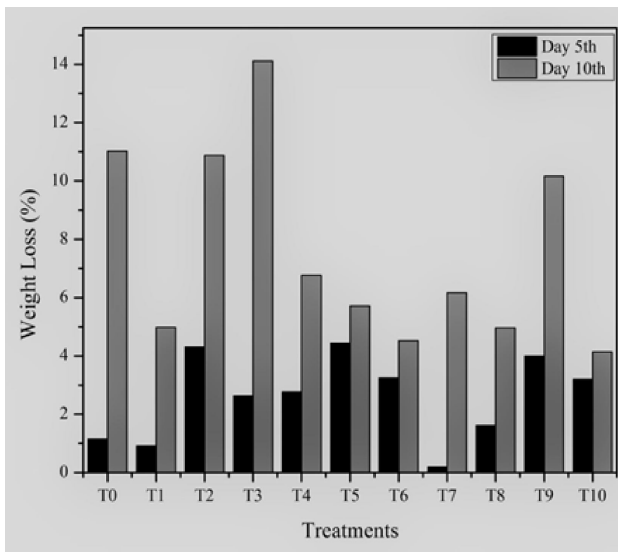


Fig. 1b: Quality index after application of essential oils : Weight loss (%)

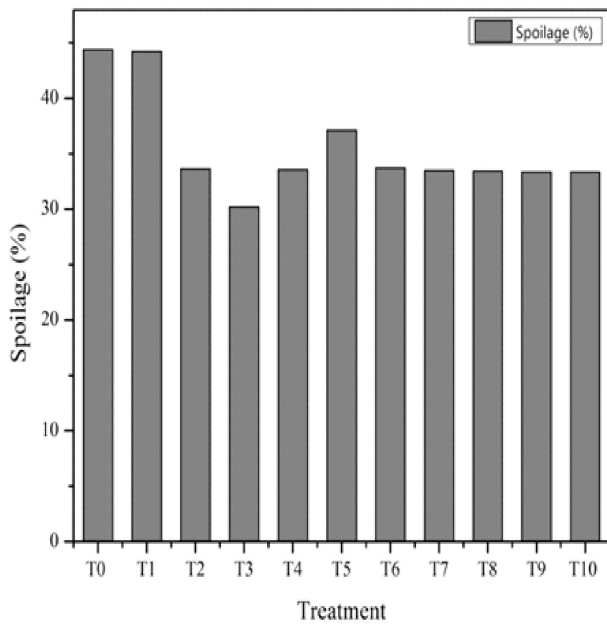


Fig. 1c: Quality index after application of essential oils : Spoilage (%)

Spoil fruit percentage

The annual spoilage of the fruits due to post harvest mishandling, storage, and supply chain decay estimated to the tune of 10 - 40% (Blackburn and Scudder, 2009). Spoilage fruit percentage of *Citrus reticulata* L. is significantly high in Sikkim. Probable reason for the high spoilage is due to high humidity prevailing in the region. Humid condition leads to maximum fruit spoilage (Moretti *et al.* 2010; Pasanen *et al.*1991). The maximum spoiled fruit percentage was observed in treatment T0 (Control) i.e. 44.40% which was found statistically at par with T1 (44.23%). The minimum spoil fruit percentage was observed in treatment T9 and T10, 33.33% which are statistically at par with T6 (33.70%), T7 (33.47%) and T8 (33.43%) Fig.1c.

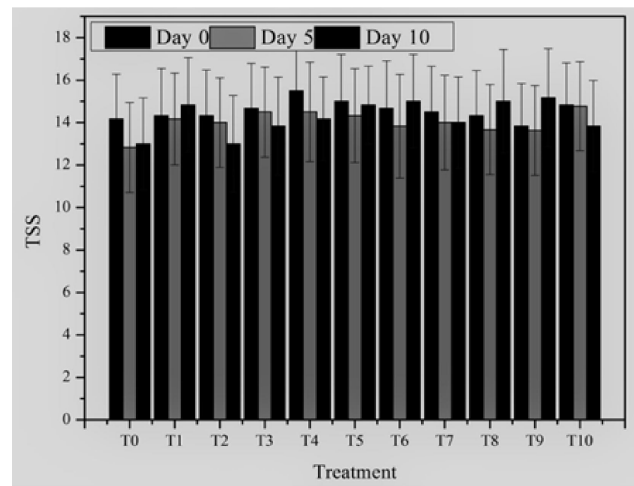


Fig. 2a: Biochemical Parameter : Total Soluble Solids (TSS)

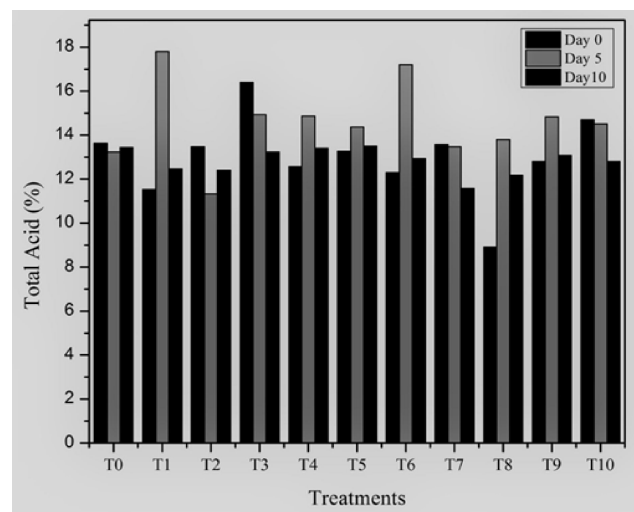


Fig. 2b: Biochemical Parameter : Total Acid(%)

Total soluble solids (TSS, °Brix)

The TSS content of a fruit includes the carbohydrates, organic acids, proteins, fats and minerals of the fruit. TSS represents 10-20% of the fruit's fresh weight and it increases as fruit matures to produce a less acidic and sweeter fruit. TSS was found to be increasing with increased storage time but dependent on the infection rate. Fruits with low degree of rotting and higher storage time were found to have maximum TSS. After five days of treatment, an initial decline was observed in TSS content in all the treatment. However, as treatment period increases to day 10 i.e. increase in storage time, all the fruits with differential essential oil treatment showed a major increase in the TSS content from the Day 0 with an exception in T0 (13.00 ± 2.12) and T10 (13.83 ± 2.16). In T0 (control) as the infection rate was higher from the treated fruits, TSS content did not increase even though the storage period was increased but in T10 although the infection rate was less than other treatment but TSS content was also found less, it may be due to the chemical reaction of the synthetic fungicide. This suggests another reason for the shift in organic base requirement. The maximum TSS was recorded in T6 (15 ± 2.21) and T8 (15 ± 2.44) and the minimum was recorded in T0 (13 ± 2.16), T2 (13 ± 2.27) and T10 (13.83 ± 2.16) (Fig. 2(a)). It suggests essential oil coated fruit will maintain its quality parameter in long storage conditions. The results are in agreement with some of the researchers who reported the increased TSS on storage after treatment with essential oils such as Anise, Ammi, Ziziphora and Cinnamon oils on peach fruit (Salem *et al.* 2016), fennel, and thyme (Abdolahi *et al.* 2010), basil, wild mint and ajowan oils on grapefruits (Biosci *et al.* 2013).

Total acid (%)

The total acid of a fruit includes all the substances of an acidic nature in the juice that react with NaOH. Citric acid is the predominating organic acid in the orange juice but other organic acids viz. malic, tartaric, benzoic and succinic acids have been reported (Miller and Rice-Evans, 1997). Therefore, titratable acidity represents all the organic acid content of a given juice. The total acidic content of fruits declines with increasing storage time. On Day 5, the average total acidic content of each treatment showed 15-26% reduction with an

exception in T1 and T6 where the initial acidic content from Day 0, T1 (11.53%) and T6 (12.3%) were increased to 17.8% and 17.2% respectively. On Day 10, each treatment showed a decline in total acid (%) by 20-30%. Interestingly, on the 10th day of storage treated fruits of T1 and T6 treatments showed ~ 1 – 2% higher acidic content from the initial value or which was found similar as that of on 0th day, suggesting no changes in total acid content (Fig. 2(b)).

In conclusion, essential oils, especially Ginger and Basil oil, may provide an alternative means of controlling postharvest green rot of citrus to increase the storage life without altering any fruit quality. This study also direct towards the use of such essential oil as promising alternative to synthetic fungicides. They can be commercially exploited and applied under storage and packaging. There is tremendous scope in exploring indigenous plant species of Himalayan region for extraction of more efficient oil. Use of essential oil in post-harvest management of citrus fruit can be a boon to the organic citrus industries.

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REFERENCES

- Abdolahi, A., Hassani, A., Ghuosta, Y., Bernousi, I. and Meshkatsadat, M.H. 2010. *In vitro* efficacy of four plant essential oils against *Botrytis cinerea* Pers.:Fr. and *Mucor piriformis* A. Fischer. *J. Essen. Oil Bearing Plants* **13**: 97–107.
- Al-Samarrai, G.F., Singh, H. and Syarhabil, M. 2013. Extracts of some plants on controlling green mold of orange and on postharvest quality parameters. *World Appl. Sci. J.* **22**: 564–570.
- Anonymous. 2014. *Comprehensive Progress Report - Sikkim Organic Mission., Annual Report.* Food Security and Agriculture Development Department, Government of Sikkim, India
- AOAC. 2016. *Official methods of analysis.* In: 20th ed. (ed. G. W. L. Jr.), AOAC, USA.
- Bakkali, F., Averbeck, S., Averbeck, D. and Idaomar, M. 2008. Biological effects of essential oils - A review. *Food Chem. Toxicol.* **46**: 446–475.
- Bhattarai, R.R., Rijal, R.K. and Mishra, P. 2013. Post-harvest losses in mandarin orange: A case study of Dhankuta District, Nepal. *African J. Agril. Res.* **8**:763–767.
- Biosci, I.J., Salimi, L., Arshad, M., Rahimi, A.R., Rokhzadi, A. and Azizi, M. 2013. Effect of some essential oils on post harvest quality of grapevine (*Vitis vinifera* cv Rasha (Siah-e-Sardasht) during cold storage. *Int. J. Bioscience.* **6**:75–83.

- Blackburn, J. and Scudder, G. 2009. Supply chain strategies for perishable products: The case of fresh produce. *Production and Operations Managtt.* **18**: 129–137.
- Inouye, S., Watanabe, M., Nishiyama, Y., Takeo, K., Akao, M. and Yamaguchi, H. 1998. Anti sporulating and respiration-inhibitory effects of essential oils on filamentous fungi. *Mycoses.* **41**: 403–410.
- Inouye, S., Abe, S., Yamaguchi and Asakura, M. 2003. Comparative study of antimicrobial and cytotoxic effects of selected essential oils by gaseous and solution contacts. *Int J Aromather.* **13**: 33-41.
- Jhalegar, M.J., Sharma, R.R. and Singh, D. 2015. *In vitro* and *in vivo* activity of essential oils against major postharvest pathogens of Kinnow (*Citrus nobilis* × *C. deliciosa*) mandarin. *J. Food Sc. Tech.* **52**: 2229–2237.
- Kouassi, K.H.S., Bajji, M. and Jijakli, H. 2012. The control of postharvest blue and green molds of citrus in relation with essential oil–wax formulations, adherence and viscosity. *Postharvest Biol. Technol.* **73**: 122–128.
- Miller, N.J. and Rice-Evans, C.A. 1997. The relative contributions of ascorbic acid and phenolic antioxidants to the total antioxidant activity of orange and apple fruit juices and blackcurrant drink. *Food Chem.* **60**: 331–337.
- Moorman G.W. and Lease R.J. 1992. Residual efficacy of fungicides used in the management of *Botrytis cinerea* on greenhouse grown geraniums. *Plant disease.* **76**: 374–376.
- Moretti, C.L., Mattos, L.M., Calbo, A.G. and Sargent, S. A. 2010. Climate changes and potential impacts on postharvest quality of fruit and vegetable crops: A review. *Food Res. Int.* **43**:1824–1832.
- Oyeleke, A. and Manga, S.B. 2008. *Essential of Laboratory Practice*, 3rd edition, Tobest Publishers, Minna, Niger State, Nigeria, 12-29.
- Pasanen, A.L., Kalliokoski, P., Pasanen, P., Jantunen, M.J. and Nevalainen, A. 1991. Laboratory studies on the relationship between fungal growth and atmospheric temperature and humidity. *Environ. Int.* **17**: 225–228.
- Pradhanang, P.M., Momol, M.T., Olson, S.M. and Jones, J.B. 2003. Effects of plant essential oils on *Ralstonia solanacearum* population density and bacterial wilt incidence in Tomato. *Pl. Disease.* **87**: 423–427.
- Robbert, A. S. and Ellen, S. A. H. 1988. *Introduction to food borne fungi*. CBS, 3rd edition. Baarn, Netherlands, 287 - 289.
- Salem, E.A., Youssef, K. and Sanzani, S.M. 2016. Evaluation of alternative means to control postharvest Rhizopus rot of peaches. *Sci. Hort.* **198**: 86–90.
- Singh, H.P., Kaur, S., Negi, K., Kumari, S., Saini, V., Batish, D.R. and Kohli, R.K. 2012. Assessment of *in vitro* antioxidant activity of essential oil of *Eucalyptus citriodora* (lemon-scented Eucalyptus; Myrtaceae) and its major constituents. *LWT - Food Sc. Technol.* **48**: 237–241.
- Someshwar, B., Israr, A. and K., T.A. 2016. Management of Soft rot (*Pythium* spp.) and Wilt (*Ralstonia solanacearum*) of Ginger (*Zingiber officinale*) in Andamans. *Vegetos- An Int. J. of Pl. Res.* **29** (special) 37–40.
- Soylu, S., Yigitbas, H., Soyly, E.M. and Kurt, a. 2007. Antifungal effects of essential oils from oregano and fennel on *Sclerotinia sclerotiorum*. *J. Appl. Microbiol.* **103**: 1021–1030.
- Vaughn, S.F., Spencer, G F. and Shasha, B.S. 1993. Volatile compounds from Raspberry and Strawberry fruit Inhibit postharvest decay fungi. *J. Food Sc.* **58**: 793–796.