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Antifungal Activity of Essential Oils Extracted from Different Plants Against *Penicillium digitatum* Causing Green Mold of Citrus

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ABSTRACT

Citrus (Citrus spp.) is a genus of the Rutaceae family that is thought to have been instigated in Southeast Asia. Citrus is number one in area and production as related to other fruits throughout the world. Punjab is a leading producer of citrus fruits. Post-harvest losses occur due to many postharvest diseases in citrus. Green mold incited by *Penicillium digitatum* is a primary destructive disease that leads to significant economic losses in citrus production. For this research, infected samples were purchased from the fruit market of Uthal, District Lasbela. *Penicillium digitatum* was identified and extracted. Healthy fruits were coated with three various essential oils concentrations of six other plant oils like olive (Oleaeuropaea L.), garlic (*Allium sativum* L.), clove (*Syzygium aromaticum* L.), neem (*Azadirachta indica* L.), castor (*Ricinus communis*), and Sesame oil (*Sesamum indicum*) were tested after inoculation with *Penicillium digitatum* on citrus cultivars and then stored in polythene bags for 4, 8 and 12 days respectively. Three replications were used in each treatment. Treatments of citrus fruits coated with at all concentrations of clove oil showed a maximum reduction of disease, i.e., 42-45% with minimum fungus growth proved to be most effective, and Sesame oil at all concentrations showed a minimum decrease of infection, i.e., 19.37% with maximum fungus growth found to be least effective after 4, 8 and 12 days individually. It is a very economical and most effective technique to control this disease.

Key words: Plant Essential Oils, *Penicillium digitatum*

INTRODUCTION

Citrus (Citrus spp.) is a member of the Rutaceae family that is thought to have originated in Southeast Asia. According to some historians, Alexander took the Citrus from India to North Africa, Turkey and Greece in the late fourth century. According to archaeologists. The origins of today's citrus varieties remain unknown, although it is well recognized that the varieties include lemon, sweet orange, pummelo, grapefruit, and lime. *Citrus aurantium* L. and *Citrus limon* (lemon) Muslims added (sour orange) to Spain, North Africa, and Syria. Following the Arab invasion, the crusaders imported citrus to Europe and began sowing seeds to spread it. Citrus reticulate was introduced into China from England in 1805, and from there, it spread across Europe. As Spain turned up its citrus-rich domains to the United States of America (USA) in 1821, the Americans took several shipments of limes, lemons, oranges, and grapefruit to New York. Railroads and oceans aided the Americans in exporting their citrus to Asia and other parts of the globe. In 1889, refrigeration engineering was deemed essential for the global exchange of citrus organic goods. Citrus Limon, commonly known as lemon and lime; *Citrus nobilis*, commonly known as the lord orange; *Citrus paradisi*, generally called grapefruit; *Citrus reticulata*, commonly known as the grapefruit tangerine; *Citrus aurantinum*, mostly called sour orange; citron is generally *Citrus medica* sweet orange is citrus sinesis. A lot of yeasts cause the decomposition of citrus fruits, and mold deterioration of citrus such as tangerine and sweet orange

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are due to Saccharomyces cervisiae and Hansenula spp (Jay *et al*., 2001).

Each kinnow tree carries about 1000 fruits (Hui 2006). Lemon (Citrus limori) is a hybrid of citron and hard orange cultivars. It is high in vitamin C and contains starch, sugar, fiber, cholesterol, calcium, iron, protein, magnesium, and foliate. Lemon natural products should be stored at 10 °C; otherwise, if left in the refrigerator for an extended period, they can lose their shape. Its juice comprises 5-6 percent citrus extract, and the acrid flavor is primarily due to the presence of citrus extract.

Pakistan is blessed with horticultural soil, making it ideal for harvesting and producing organic products. Citrus is the most important organic crop being produced in Pakistan, so it's worth mentioning. Its production reaches 1,832 thousand tonnes each year, and its trade 493 tonnes worth approximately $24,500 million. After mango, banana, grapes, and apple, citrus is Pakistan's most common food. In terms of global ranking, Brazil is the first country in citrus production, and Pakistan is thirteenth in citrus production and falls sixth in the production of kinnow. Ninety-five percent of the whole creation is explicitly delivered in the Punjab district. The Punjab district includes Sargodha, and Faisalabad, Layyah, Sahiwal having different varieties of citrus-like kinnow, manderian and fruiter early, which are Pakistan's prominent citrus growing areas.

Problem Area

The postharvest diseases of citrus fruits due to fungi natural products include anthracnose (*Colletotrichum gloeosporioides*), spurrot (*Geotrichum candidum*), and brown decay (*Phytophthora citrophthora*) (Iqbal, 1996), and green mold *Penicillium digitatum*. The fundamental causes of such problems are poor and inadequate storage conditions. Monilinia, Penicillium spp., Aspergillus, Alternariaspp., Rhizopusstolonifer, and Botrytis cinerea are the most prevalent parasitic pathogens that cause postharvest diseases (Ogawa *et al*., 1995). Out of these, *Penicillium digitatum* (Green mold) is almost found in each citrus orchard and is active in the prevailing season, which becomes rotten. It is the leading and significant factor that causes losses of billion dollars during post-harvest (Pitt, 1981).

Penicillium is the postharvest pathogen that causes fruit decay. The pathogens *P. digitatum*, which produces green mold is the most prevalent on citrus fruits. *P. digitatum* is the more prevalent pathogen. *P. digitatum* boosts ethylene production, which speeds up the maturation of damaged fruits (Kirk *et al*., 2008).

It is assumed that one-fourth of the creation would not hit the consumer until harvest, leaving all of the capital and time spent on the product worthless. As a result, both consumers and manufacturers would need to reduce and eliminate such waste. It is surprising and unsettling to hear that so much money was spent on the water system, seed assurance, and treatment and that so much time was spent cultivating the plant was squandered and lost just seven days after harvest. It's also true that the fruit of citrus, among many crops, is affected most due to diseases that appear after harvest. As a consequence, postharvest management must be given the same significant yield approaches. Nutritional security can be achieved by growing crop yields and limiting post-harvest losses. Pathogens cause 20-25 percent of citrus fruit to be lost after harvesting in developing countries while controlling the losses and different tasks after harvesting (Droby, 2005; Zhu, 2006). Due to insufficient transportation and capacity, postharvest losses in vegetables and natural products are 25-40 %.

Currently, it is a critical method that is introduced to replace progressive control strategies. Because of the excellent decline in pesticide viability amid astounding reductions in desirable fungicides, as well as recent concerns regarding the natural effects of using fungicides as a consequence of ongoing European regulations, there has been seen limited usage of conventional fungicides. Awareness of food and nutrition assurance and environmental factors has been introduced in the area of palatable coatings. The resources needed to refuel the formation of covering layers are readily available (Kim *et al*., 2003).

Efficacy of different plant oils like neem clove and eucalyptus were used against two Penicillium species at a concentration of 500 ppm, while 1000 ppm convergence of thyme pith causes the Alternaria mycelium's production restriction to be lifted. The experts declared the hindrance request of the concentrates they were thinking of, such as 'thyme cumin mint' (Azizi *et al*., 2007).

Clove seems to be the most critical oil, according to the findings of various inquiries. This basic oil was put to the test against ten diseases after harvesting, which increased causes spread. The fixation range used in this study was 100 to 1000 ppm, with 700 ppm causing spore germination to be hindered and 300 ppm causing spore restriction in most organisms (70 to 100 percent). Several citrus postharvest pathogens, such as *A. citri, P. italicum, and P. digitatum* were studied using oils from *C. basilicum, T. vulgaris, M. piperita*, and *S. hortensis*. These studies showed that at 500 ppm, T. vulgaris specific oil inhibits mycelium production of two forms of Penicillium (Azizi *et al*., 2007).

These damages can be avoided if harvests, especially vegetables and fruits, are adequately cared for during handling. Because of their flavor and taste, frozen orange juices attract a lot of attention on the globe. The green type (*P. digitatum*) and blue form (*P. italicum*) of mandarin orange postharvest disease caused the most severe losses (Prabakar *et al*., 2004). To ensure the protection of the food supply, it is critical to limit citrus organic crop losses caused by postharvest diseases. Legal dealings and drugs will help to mitigate these misfortunes to a large extent. The recent study was planned to investigate the myco flora of postharvest citrus leafy foods. The executives of P digitatum (green form) treated the organic products with various oil covering was applied at different fixations to control the green shape *Penicillium digitatum*.

It is dynamic to reduce the damage of citrus fruit caused by post-harvest disease to confirm food supply protection. These losses can be decreased to be considerable level through appropriate treatment and handling. Keeping in view the destructive nature of the pathogen and the extent of losses recent study was planned with the following objectives.

Objectives

To find out the resistant citrus fruits cultivars

To evaluate the most effective essential oil for the management of green mold of citrus caused by *Peniclillium digitatum.*

MATERIALS AND METHODS

Collection of Diseased Samples

Surveys were conducted for the collection of diseased samples of citrus fruit from the different markets and fruit shops of Uthal, District Lasbela. Affected citrus fruits showing typical disease symptoms of green mold were examined and selected as samples. These samples were collected in polythene bags and brought to Plant Pathology Laboratory, Faculty of Agriculture, LUAWMS, Uthal for further study.

Isolation, identification and purification of Penicillium digitatum

Isolation techniques were followed to obtain *Penicillium digitatum* culture. About 1-2 cm diseased portion of rotten citrus were cut, surface sterilized with 70% ethyl alcohol, rinsed twice in distilled water, and dried with filter paper. Then surface sterilized samples were placed on autoclaved solidified potato dextrose agar (PDA) contained in sterilized Petri plates. These Petri plates were incubated at room temperature (25±2 0C). After 5-7 days, the fungal growth that appeared on diseased samples were identified and transferred to potato dextrose agar slants for further purification process. The pure culture of *P. digitatum* was preserved in the refrigerator at 4 0C and sub-cultured periodically for further study.

Pathogenicity Test

Pathogenicity test were carried out in the Plant Pathology Laboratory, Faculty of Agriculture, LUAWMS under a completely randomized design (CRD). Tap water was used for washing healthy and fresh citrus. Then the surface was disinfected with 0.1% sodium hypochlorite solution for 1-2 minutes followed by rinsing twice in sterilized distilled water and finally dried with filter paper to avoid any contamination and excessive moisture. After making 4-5 injuries on citrus fruits like Musambi, Lemon and grapefruit in 1cm circle with the help of a sterilized sharp scalpel inoculated with isolated fungus was carried out. Control was used without injuries having no inoculum. There were three replications of each treatment in each replication five citrus fruits were used. After this inoculated citrus with or without injuries having no inoculum were kept at room temperature under bell jar or in polythene bags. Observations were made for symptoms development periodically according to the disease rating scale (Cuero *et al*., 1987). Re-isolation was made from inoculated citrus fruit and the culture thus obtained from diseased samples were compared with the original culture to confirm the identity and pathogenicity of the pathogen.

Screening of Citrus Fruit Cultivars against Green Mold of Citrus

In this trial different citrus fruit cultivars like Fruiter early, Musambi, Lemon, Grape fruit and Sweet orange were evaluated against green mold of citrus in the laboratory under CRD to check their level of resistance. Purified culture of isolated fungus for inoculation was made on (PDA) medium. Fresh fruit of different citrus cultivars as mentioned earlier were injured 4-5 times and made holes in 1cm circle with help of sterilized needle. Circles were made with the help of marker, then isolated fungus was applied in these holes with help of a contaminated needle. There were three replications of each treatment in each replication five citrus fruits will be used and these samples will be placed in polythene bags and were remained at room temperature. Symptoms were checked after week. The seeing results were assessed on the basis of disease rating scale described by Cuero *et al*. (1987) mentioned as above.

Evaluation of the Antifungal Activity of Essential Oils against Green Mold of Citrus

This experiment was carried out in the laboratory conditions under CRD. In this trial different cultivars of citrus fruits like Fruiter early, Musambi, Lemon, Grape fruit and Sweet orange were treated with three different concentrations i.e. 30%, 60%, 90% of different plant essential or edibles oils like Sesame (*Sesamum indicum* L.), caster (*Ricinus communis* L.), olive (*Olea europaea* L.), garlic (*Allium sativum* L.), clove (*Syzygium aromaticum* L.) and neem (*Azadirachta indica* L.) against green mold of citrus incited by *Penicillium digitatum* to determine their role in case of shelf life of citrus fruits. For making 30% concentration 30ml of oil was added in 70 ml of ethanol. Similarly, for 60% and 90% concentration 60ml and 90 ml oil was added in 40 ml and 10ml of ethanol respectively.

Fresh fruit of different cultivars as mentioned above were injured with the help of needle in 1cm circular shape and marked with a permanent marker. Then applied fungus with the help of contaminated needle from the Petri plates containing 7 days old culture of target fungus. After this covered the whole surfaces of the inoculated citrus fruits by coating them with three different concentrations i.e. 30%, 60%, 90% of different plant essential or edibles oils as mentioned above respectively. Inoculated citrus fruits without coating of different plant essential oils were used as control. There were three replications of each treatment were used. All this material was placed in polythene bags and rotting symptoms were checked after 4, 8 and 12 days' intervals according to the disease rating scale of (Cuero *et al*., 1987).

Coating

Fresh fruit had been damaged and tagged with a persistent marker using a circular needle. Then, using a contaminated needle from the Petri dish, I administered the fungus. Afterward, using the most efficient E. oil dosages, coat the thought the entire top of the fruit. All of these samples were stored in polyethylene bags, and decaying indications.

Data was calculated after 4, 8, and 12 days by the following given formula:

$$\textbf{\textit{Percent Disease Control}} = \frac{\textbf{\textit{Control}} - \textbf{\textit{Treatment}}}{\textbf{\textit{Control}}} \times \mathbf{100}$$

Statistical Analysis

All procedures, including such citrus species screenings and infection control with various oils, were conducted in a lab and were organized adopting a completely randomized design (CRD). The statistical analyses were performed by Statistix 8.1. The statistical differences were considered significant at the $P< 0.05$ level (Steel and Torrie 1997).

RESULTS AND DISCUSSION

Collection of Diseased Samples and Identification of the Pathogen

In Pakistan, there is a wide range of citrus varieties that are extensively produced. The susceptibility of different kinds of infection may differ from each other. When it relates to citrus fruit, the most prominent finding is its great sensitivity to other diseases, whether it establishes tolerance to one. To analyze the influence of a particular disease, it is necessary to screen a significant number of citrus cultivars towards post-harvest. It is critical to identify and infect the fruits with such a particular fungus during the treatment of fungal infections. Our study was carried out in order to collect damaged citrus fruit samples from Uthal's marketplaces and fruit stores. Citrus fruits with characteristic green mold infection symptoms were evaluated and recommended as samples. Collected materials were gathered in polythene bags and agreed to send to the Plant Pathology Laboratory of the Faculty of Agriculture, LUAWMS, Uthal, for further analysis. *Penicillium digitatum* was initially regarded as the source of rotting in this disease. Fruits infected with the fungus were isolated and sanitized. In order to infect healthy fruits in an experiment, this fungus was required. Following correct identification, pure culture was formed following inoculum multiplication.

Screening of Different Citrus Cultivars

In the screening trial, different cultivars of citrus fruits were tested for infection with varying species of fungus and postharvest rotting. The cultivars used included Musambi, Sweet orange, Lemon, and Grapefruit. Three fungi, *Aspergillus niger*, Penicillium digitatum, and *Penicillium italicum*, were isolated from citrus cultivars during the screening trial having *Penicillium italicum* isolated from all varieties except Grapefruit and *Aspergillius niger* isolation from lemon and orange and other two cultivars showed resistance response against this fungus Table 2.

Response of Various Cultivars of Citrus Fruit and Pathogenicity after Inoculation

The results showed in the Table (2) after 5 days showing *Penicillium digitatum* was found in all kinds of citrus fruit. It was isolated from all cultivars, but *Penicillium italicum* was not found in musambi and Grapefruit. *Aspergillius niger* was only separated from sweet orange cultivars, while the remaining verities showed resistant behavior against the fungus.

The highly resistant response was noticed in the case of Grapefruit as Penicillium digitatum, which showed an area of 2 cm that comprised less than ¼ of the fruit area and decreased green mold growth. The highly susceptible response was noticed in the case of Sweet orange as *Penicillium digitatum* showed an increase over an area of 13 cm2 which comprised more than half of the extent of fruit. Lemon also showed a susceptible response due to high green mold growth, and *Penicillium digitatum* showed growth an area of 7 cm2 which comprised less than ¾ and more ½ of the fruit area. The susceptible response was also noticed in the case of Musambi orange as it showed green mold disease and growth of *Penicillium digitatum* covered an area of 7 cm2 which comprised less than ¾ and more ½ of the fruit area. In the pathogenicity test after inoculation *P. digitatum* was re-isolated and confirmed by Koch's postulates (Table 3).

Management through Essentials Oils

Six different plant oils were evaluated against fungal development on citrus cultivars, including olive (*O. europaea* L.), garlic (*A. sativum* L.), clove (*S. aromaticum* L.), neem (*A. indica* L.), castor, and Sesame oil. Were tested at three different concentrations i.e. 30%,60%,90%, against this fungus on citrus cultivars respectively.

Effect of Plant Essential Oils Coating on Grapefruits

Table (4) Clove oil showed the highest disease elimination, i.e., 42.45 % with the minimal possible of fungus development, and 30 % Sesame oil indicated the most disease decline, i.e., 90 % 19.37 % with the least amount of fungus development after four days. Results also showed that increasing the concentration of plant oils at 30% controlled the fungus growth in grapefruit. Recorded data shows that clove oil at 90% concentration is best for the reduction of Penicillium digitatum.

The data was recorded for three different concentrations of plant oils, i.e., 30%, 60%, and 90%. Afterward, statically data showed disease reduction by increasing the concentration of clove oil from 30% to 90% (26.95%, 34.47%, and 42.45 %.) separately Table (4) shows that there were significant differences among different essential oils.

Table (6) shows Clove oil treatment 90% clove oil reduced disease by 62.35 % with no fungus development, whereas 30% Sesame oil reduced disease by 42.93 % with no fungus growth after 8 days. Results also showed that by increasing plant oils that control the fungus growth in grapefruit recorded data, clove oil was found best for reducing the pathogen in the Table (6).

Three different concentrations of plant oils, i.e., 30%, 60%, and 90%, showed disease reduction improvement by increasing the concentration of clove oil from 30% to 90% 47.43%, 55.47%, and 62.35%, respectively, data showed that significant relationship exists between different concentrations of different plant oils.

Table 8 indicates that treating fruits using 90 % cloves oil resulted in a maximum disease decrease of 83.06 % with minor fungus development after 12 days. However, treatment fruits using 30 % Sesame oil ultimately results in a low disease reduction of 56.13 % with maximal fungus expansion. In addition, enhancing the level of plant oils suppresses fungal formation, according to the study results. According to the findings, clove oil at a dosage of 90% was displayed to be the most efficacious in decreasing the production of *Penicillium digitatum* in grapefruit.

The data was recorded for three different concentrations of plant oils, i.e., 30%, 60%, and 90%, and expressed disease reduction by increasing concentration of clove oil from 30% to 90% 62.20%, 73.05%, and 83.06%. Individually, they stated that there was a significant association across various plant oil concentrations. Furthermore, the findings of cultivar grapefruit proved that *Penicillium digitatum* seemed to have the best control on post-harvest rotations. The optimum outcomes were achieved by incorporating a higher concentration of plant oil into grapefruit.

Table 1: Disease Rating Scale

Score	Symptoms	Remarks
0	No visible symptoms	Immune
1	Mold growth covered less than ¼ of citrus surface	Resistant
2	Mold growth covered greater than ¼ but less than ½ of citrus surface	Moderately Resistant
3	mold growth covered ½ or more but less than ¾ of citrus surface	Susceptible
4	mold growth covered ¾ or more of citrus surface	Highly Susceptible

(Cuero *et al*., 1987)

Table 2: Association of postharvest fungi with different citrus cultivars

Pathogen	MUSAMBI	SWEET ORANGE	LEMON	GRAPEFRUIT
Penicillium digitatum (Green Mould)	+	+	+	+
Penicillium italicum (Blue Mould)	-	+	+	-
Aspergillus niger (Black Mould)	-	+	-	-

+= Isolated - = Not isolated

Table 3: Response of different citrus varieties of citrus fruit after artificial inoculation of Penicillium digitatum

Name of cultivar	Symptoms existence	Rating Score	Response
Grapefruit	Green progress indicated with about 2 cm shielded area. (less than ¼ of fruit surface)	1	Resistant
Lemon	Green development revealed with about 7 cm enclosed area. (more than ½ and less than ¾ of fruit surface)	3	Susceptible
Musambi	Green growth appeared with about 7 cm covered area. (more than ½ and less than ¾ of fruit surface)	3	Susceptible
Sweet orange	Green growth appeared with about 13 cm covered area. (more than ½ of fruit surface)	4	Highly Susceptible

Fig. 1: Fungal growth on the surface of different citrus cultivars during screening trial.

The results of a recent study are in line with the results found by Levinskaite (2012). They found that clove oil is best against the growth of Green mold (Penicillium digitatum). The same findings were described by Chang *et al*. (2008) that clove oil is the best alternative treatment to reduce fungus growth. They found that clove oil is safe to use and gives good results regarding control of green mold.

Effect of Plant Essential Oils Coating on Musambi

Table (10) shows a significant relationship between the different concentrations of plant oils. Clove oil showed a maximum reduction of disease of 40.51% with minimum fungus growth, and Sesame oil showed the most negligible reduction of disease, 16.33% at 30% with maximum fungus growth after 4 days. Results showed that by increasing the concentration of plant oils which control the fungus growth. It is clear from recorded data that clove oil at 90% concentration was observed best to reduce fungus growth on musambi.

Statically the data was recorded for three different concentrations of plant oils, i.e., 30%, 60%, and 90% expressed disease reduction improvement by increasing concentration of clove oil from 30% to 90% (24.14%, 32.41%, and 40.51%).

The results expressed in (Table 12) that There was a substantial difference between essential oils, with clove oil revealing the greatest decrease in infection at 90%. 64.37% had the least amount of fungal development, while 30% had the least amount of diseases elimination after 8 days. Fungus growth is at its peak in 43.66 % of cases.

Table 4: Evaluation of essential oils coated on grapefruit after four days

	Grape Fruit Treatment			
Extracts	30%	60%	90%	Means
Clove oil	26.95 a	34.47 a	42.45 a	34.62
Neem oil	24.88 bc	32.06 bc	40.45 bc	32.46
Garlic oil	26.35 ab	33.18 ab	41.44 ab	33.65
Castor oil	22.07 d	29.34 d	38.24 d	29.88
Olive oil	23.54 cd	30.40 cd	39.13 cd	31.02
Sesame oil	19.37 e	28.49 d	37.85 d	28.57
MEANS	23.86	31.32	39.93	

Table 5: Analysis of Variance for fruit coatings with essential oils on grapefruit

Sources	DF	SS	MS	F	P
Dose	2	1926.28	963.13	3354.37	0.0000
Treatment	5	202.04	40.40	140.73	0.0000
Dose*Treatment	10	5.09	0.509	1.77	0.1017
Error	36	10.34	0.287		
Total	53	2143.74			

Table 6: Analysis of Variance for with essential oils coated on grapefruit

Sources	DF	SS	MS	F	P
Dose	2	3826.01	1913.01	26992.1	0.0000
Treatment	5	279.90	55.98	789.86	0.0000
Dose*Treatment	10	7.22	0.72	10.19	0.0000
Error	36	2.55	0.07		
Total	53	4115.68			

Table 7: Evaluation of essential oils coated on grapefruit after 12 days

	Grape Fruit			
Treatment	30%	60%	90%	Means
Clove oil	62.20a	73.05a	83.06a	72.77
Garlic oil	60.08b	71.35b	81.33b	70.92
Neem oil	59.34c	70.32c	80.10c	69.92
Olive oil	58.15d	69.54c	79.07d	68.92
Castor oil	57.07e	67.17d	78.02e	67.42
Sesame oil	56.13f	66.13e	75.07f	65.77
Means	58.83	69.59	79.44	

Table 8: Analysis of Variance for fruit coating with essential oils on Musambi

Sources	DF	SS	MS	F	P
Dose	2	2718.71	1559.31	2988.29	0.0000
Treatment	5	312.71	62.54	137.49	0.0000
Dose*Treatment	10	106.69	10.67	23.45	0.0000
Error	36	16.38	0.45		
Total	53	3154.48			

Table 9: Evaluation of essential oils coated on Musambi after four days

	Musambi Concentrations			
Extracts	30%	60%	90%	
Clove oil	24.14 a	32.41 a	40.51 a	32.35
Neem oil	20.67bc	29.44 b	38.42bc	29.51
Garlic oil	22.33ab	30.45 b	39.50 ab	30.76
Castor oil	17.70d	25.66d	35.20d	26.18
Olive oil	18.59cd	27.22 c	37.50c	27.77
Sesame oil	16.33d	23.33e	32.84 e	24.16
Means	19.96	28.08	37.33	

Results also indicated that increasing the concentration of plant oils that control fungus growth in musambi recorded data that clove oil at 90% concentration managed best to reduce the target pathogen.

Table 10: Analysis of Variance for fruit coatings with essential oils on Musambi

Sources	DF	SS	MS	F	P
Dose	2	1943.10	971.54	4304.53	0.0000
Treatment	5	197.48	39.49	174.99	0.0000
Dose*Treatment	10	3.89	0.389	1.73	0.1126
Error	36	8.13	0.226		
Total	53	2152.60			

Table 11: Evaluation of essential oils coated on Musambi after eight days

	Musambi Concentrations			
Extracts	30%	60%	90%	
Clove oil	49.47a	58.04a	64.37a	57.29
Neem oil	47.22bc	56.08bc	61.41bc	54.90
Garlic oil	47.83b	57.22ab	62.41b	55.82
Castor oil	44.22d	54.34de	59.37de	52.64
Olive oil	46.30c	55.30cd	60.24cd	53.94
Sesame oil	43.66d	53.06e	57.99e	51.57
Means	46.45	55.67	60.97	

Table 12: Analysis of Variance for fruit coatings with essential oils coated on Musambi

Sources	DF	SS	MS	F	P
Dose	2	4003.73	2001.87	26150.6	0.0000
Treatment	5	227.78	45.56	595.11	0.0000
Dose*Treatment	10	2.65	0.26	3.46	0.0029
Error	36	2.76	0.08		
Total	53	4236.93			

Table 13: Evaluation of essential oils coated on Musambi after 12 days

	Musambi Concentrations			
Extracts	30%	60%	90%	
Clove oil	61.37a	72.15a	82.01a	71.84
Neem oil	58.26c	69.53b	79.68b	69.15
Garlic oil	59.17b	70.35b	80.25b	69.92
Castor oil	56.49d	67.39c	77.56d	67.14
Olive oil	57.03d	68.19c	78.26c	67.82
Sesame oil	55.09e	65.03d	76.17e	65.43
Means	57.90	68.77	78.99	

Table 14: Analysis of Variance for fruit coatings with essential oils coated on Lemon

Sources	DF	SS	MS	F	P
Dose	2	2280.92	1140.27	2235.96	0.0000
Treatment	5	256.78	51.36	100.69	0.0000
Dose*Treatment	10	27.76	2.78	5.44	0.001
Error	36	18.36	0.51		
Total	53	2583.82			

Table 15: Evaluation of essential oils coated on Lemon after four day

	Lemon Concentrations			
Extracts	30%	60%	90%	Mean
Clove oil	27.55a	34.47a	41.79a	34.60
Neem oil	24.21c	32.06bc	39.46 abc	31.91
Garlic oil	25.69b	33.18ab	40.48 ab	33.11
Castor oil	20.73e	29.34d	37.85 cd	29.88
Olive oil	22.86d	30.40cd	38.79 bcd	30.68
Sesame oil	18.70f	28.49d	36.91 d	28.03
Means	23.29	31.32	39.21	

Three different concentrations of plant oils are 30%, 60% and 90%. Showed disease reduction statically by increasing concentration of clove oil from 30% to 90%

Table 16: Analysis of Variance for fruit coatings with essential oils coated on Lemon

Sources	DF	SS	MS	F	P
Dose	2	1386.92	693.46	6319.46	0.0000
Treatment	5	204.35	40.87	372.45	0.0000
Dose*Treatment	10	48.36	4.836	44.07	0.0000
Error	36	3.95	0.110		
Total	53	1643.58			

Table 17: Evaluation of essential oils coated on Lemon after eight days

Lemon				
Concentrations				
Extracts	30%	60%	90%	Mean
Clove oil	48.25a	56.08a	61.12a	55.15
Neem oil	45.13c	53.61b	57.07c	51.93
Garlic oil	44.83b	54.36b	58.12b	52.43
Castor oil	43.02d	51.16cd	55.07e	49.75
Olive oil	44.43c	52.20c	56.16d	50.93
Sesame oil	41.17d	50.50d	54.36f	48.67
Means	44.81	52.99	56.98	

Table 18: Analysis of Variance for fruit coatings with essential oils coated on Lemon

Sources	DF	SS	MS	F	P
Dose	2	3672.64	1836.32	16358.1	0.0000
Treatment	5	221.46	44.29	394.55	0.0000
Dose*Treatment	10	3.22	0.32	2.87	0.0098
Error	36	4.04	0.11		
Total	53	3901.35			

Table 19: Evaluation of essential oils coated on Lemon after 12 days

Lemon				
Concentrations				
Extracts	30%	60%	90%	mean
Clove oil	60.40a	71.02a	80.16a	70.52
Neem oil	57.27c	68.49c	77.34c	67.7
Garlic oil	58.40b	69.47b	78.31b	68.72
Castor oil	55.37d	66.66d	75.33e	65.78
Olive oil	56.27cd	67.69c	76.28d	66.74
Sesame oil	53.05e	65.42e	74.24f	64.23
Means	56.79	68.13	76.94	

Table 20: Analysis of Variance for fruit coatings with essential oils coated on Sweet orange

Sources	DF	SS	MS	F	P
Dose	2	2960.82	1480.41	5240.39	0.0000
Treatment	5	450.20	90.04	318.72	0.0000
Dose*Treatment	10	30.94	3.09	10.95	0.0000
Error	36	10.17	0.28		
Total	53	3449.13			

Table 21: Evaluation of essential oils coated on Sweet orange after four days

Sweet orange				
Concentrations				
Extracts	30%	60%	90%	Mean
Clove oil	24.67a	33.08a	42.17a	33.30
Neem oil	21.34b	28.77c	38.42c	29.51
Garlic oil	22.52b	31.11b	40.17b	31.26
Castor oil	16.37d	27.37c	35.87d	26.53
Olive oil	18.59c	28.33c	37.50cd	28.14
Sesame oil	14.99e	24.99d	33.18e	24.38
Means	19.75	28.94	37.88	

(49.47%, 58.04% and 64.37 %), respectively. The data showed that a significant relationship exists between different concentrations of plant oils.

Table 22: Analysis of Variance for fruit coatings with essential oils coated on Sweet orange

Sources	DF	SS	MS	F	P
Dose	2	2149.85	1074.93	10559.5	0.0000
Treatment	5	203.81	40.76	400.43	0.0000
Dose*Treatment	10	4.62	0.46	4.54	0.0004
Error	36	3.66	0.10		
Total	53	2361.95			

Table 23: Evaluation of essential oils coated on Sweet orange after eight days

Sweet orange				
Concentrations				
Extracts	30%	60%	90%	Mean
Clove oil	50.08a	59.50a	65.55a	58.37
Neem oil	47.33c	56.17c	62.05c	55.18
Garlic oil	48.26b	57.48b	63.10b	56.28
Castor oil	45.01d	54.35d	61.28c	53.54
Olive oil	46.43c	55.10d	61.32c	54.28
Sesame oil	44.05e	52.92e	60.11d	52.36
Means	46.86	55.92	62.23	

Table 24: Analysis of Variance for fruit coatings with essential oils coated on Sweet orange

Sources	DF	SS	MS	F	P
Dose	2	4019.38	2009.69	21572.6	0.0000
Treatment	5	310.15	62.03	665.84	0.0000
Dose*Treatment	10	2.98	0.30	3.19	0.0050
Error	36	3.35	0.09		
Total	53	4335.82			

Table 25: Evaluation of essential oils coated on Sweet orange after 12 days

Sweet orange				
Concentrations				
Extracts	30%	60%	90%	Mean
Clove oil	63.15a	73.30a	84.40a	73.61
Neem oil	60.33c	70.27c	81.17c	70.59
Garlic oil	61.35b	71.59b	82.24b	71.72
Castor oil	57.49d	68.05d	78.45e	67.99
Olive oil	58.38d	69.57c	79.39d	69.11
Sesame oil	55.39e	66.42e	77.24f	66.35
Means	59.35	69.86	80.48	

Statistical results in a Table (14) found that 90% clove oil indicated the maximum reduction of disease 82.01% with minimum fungus growth, and Sesame oil showed the least reduction of disease 55.09% at 30% with maximum fungus growth after 12 days. Results also showed that by increasing the concentration of plant oils which control the fungus growth. It is clear from recorded data that clove oil at 90% concentration best reduces fungus growth on fruit.

The data was recorded for three different concentrations of plant oils 30%, 60%, and 90%. Afterward, the complete randomized design was applied to the information, which showed disease reduction by increasing the concentration of clove oil from 30% to 90% (61.37%, 72.15%, and 82.01%) respectively, the data showed that a significant relationship exists between different concentrations of various plant oils.

It was found that clove oil proved good efficacy against the fungus growth, particularly *Penicillium digitatum* and *P. italicum*. The results are also in line with the findings of Anwar and Chaudhary (2004). They found the effectiveness of some plant oils, including clove oils, against fungus growth on citrus fruit.

Effect of Plant Essential Oils Coating on Lemon

Table (16) shows a significant difference between different essential oils. Clove oil showed a maximum reduction of disease of 41.79% with lowest growth of the fungus and 30% whereas, Sesame oil indicated the minimum decline of disease by 18.70% with maximum fungus growth after 4 days. Results also showed that by increasing the concentration of plant oils which control the fungus growth. In lemon recorded data, clove oil at 90% concentration controlled best for reducing *P. digitatum*.

The data was recorded for three different concentrations of plant oils, i.e., 30%, 60%, and 90% showed disease reduction improvement by increasing concentration of clove oil from 30% to 90% (27.55%, 34.47%, and 41.79%) separately after 4 days.

Statistical results in Table (18) show that a significant relationship exists between different concentrations of plant essential oils the concentration 90% of clove oil revealed a high level of disease reduction, 61.12% with minimum fungus growth, and Sesame oil showed a minor reduction of disease 41.17%, at the 30% with maximum fungus growth after 8 days. Results also showed that increasing the concentration of plant oils controlled the fungus growth. Recorded data shows that clove oil at 90% concentration was found best to reduce the pathogen in lemon.

The data was recorded for three different concentrations of plant oils, i.e., 30%, 60%, and 90% showed disease reduction by increasing attention of clove oil from 30% to 90% (48.25%, 56.08%, and 61.12%) individually after 8 days.

Table (20) demonstrates that fruits containing 90% clove oil had the most diseases control (80.16%) with the least amount of fungus growth, while Sesame oil had the least amount of disease reduction (53.05% at 30%) with the highest level of fungus development. Results also expressed that by increasing the concentration of plant oils which control the fungus growth. It is clear from recorded data that clove oil at 90% concentration was found best to reuse the development of *P. digitatum* on the lemon.

The data was recorded for three different concentrations of plant oils, i.e., 30%, 60%, and 90%, showed disease reduction improvement by increasing concentration of clove oil from 30% to 90% (60.40%, 71.02%, and 80.16%) respectively. The data statically expressed that a significant relationship exists between different concentrations of different plant oils after 12 days.

The current study's findings are in line with the results of Yahyazadeh *et al*. (2008). They found that clove oil proved good efficacy in the inhibition of fungal growth. The oil inhibited the growth of *P. digitatum* on citrus fruit. Clove oil, cinnamon leaf oil, mustard oil, and orange oil were effective against fungus growth.

Effect of Plant Essential Oils Coating on Sweet Orange

Table (22) shows a significant relationship exists between the different concentrations of plant oils. Clove oil showed a maximum reduction of disease 42.17% at 90%with minimum fungus growth, and Sesame oil showed a minimum reduction of disease i 14.99% at 30% with maximum fungus growth. Results also showed that increasing the concentration of plant oils controls fungus growth. It is clear from recorded data that clove oil at 90% concentration was observed best to reduce fungus growth sweet orange after 4 days.

Statically data was recorded for three different concentrations of plant oils, i.e., 30%, 60%, and 90%, and expressed disease reduction improvement by increasing the concentration of clove oil from 30% to 90% (24.67%, 33.08%, and 42.17%) separately. The results in Table (24) expressed a significant difference among different essential oils at 90% clove oil showed a maximum reduction of disease 65.55% with minimum fungus growth and 30% Sesame oil showed least reduction of disease 44.05% with maximum fungus growth. Results also showed that by increasing the concentration of plant oils which control fungus growth. It is clear from recorded data that clove oil at 90% concentration controlled was best for reducing target pathogen after 8 days.

Three different concentrations of plant oils are 30%, 60%, and 90%. Statically showed disease reduction improvement by increasing concentration of clove oil from 30% to 90%, (50.08%, 59.50%, and 65.55%)respectively Statically results in Table (26) shows that 90% clove oil indicated a maximum reduction of disease 84.40% with minimum fungus growth and Sesame oil showed the least reduction of disease 55.39% at 30% with maximum fungus growth. Results also showed that by increasing the concentration of plant oils which control the fungus growth. It is clear from recorded data that clove oil at 90% concentration controlled was best to reduce fungus growth on fruit.

The data was recorded for three different concentrations of plant oils, i.e., 30%, 60%, and 90% showed disease reduction improvement by increasing concentration of clove oil from 30% to 90% (63.15%, 73.30%, and 84.40%) separately. The data showed that a significant relationship exists between different concentrations of various plant oils after 12 days. Similar findings were also observed by Hui (2006). They found antifungal effects of cinnamon and clove oil against *Aspergillus niger*, *A. oryzae* and *A. ochraceus*.

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